Endophytes in Maize (*Zea mays*) in New Zealand

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Jennifer Joy Brookes

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The aim of this study was to isolate fungal endophytes from maize in New Zealand (NZ) and to select endophytes with potential to reduce insect pests and/or plant diseases. Culture methods were used to isolate 322 isolates of fungi belonging to four phyla from maize (*Zea mays* L.) plants. Plants were sampled over two growing seasons (2014 and 2015) in two regions of NZ. Morphological and molecular (ITS rDNA sequencing) techniques were used to identify the fungi. The most common genera recovered were *Fusarium*, followed by *Alternaria, Trichoderma, Epicoccum, Mucor, Penicillium* and *Cladosoprium* spp. Of the Acomycota isolates, 33 genera from 6 classes were recovered. Basidiomyctes were represented by two classes and Zycomycota by one class and a superphylum, Heterokonta, was represented with one class. To determine fungi with potential as biocontrol agents, several assay approaches were taken. Initially, most fungi were used to challenge a plant pathogen on media plates in dual culture experiments. This allowed selection of 21 promising isolates which were inoculated into maize plants by seed coating, then used in plant disease assay and a caterpillar feeding challenge assay.

A final eight isolates were selected as the most promising for conferring beneficial traits on plants: *Sordaria fimicola, Mucor racemosus, Mucor fragilis, Trichoderma atroviride, Penicillium brasilianum, Fusarium equiseti, Fusarium acuminatum* and *Fusarium proliferatum.* These isolates have shown potential as BCAs against disease and/or insects in laboratory assays.

Keywords: Endophytes, BCA, maize, fungi, screening, bioassay, dual culture, *in planta, Setosphaeria turcica, Helicoverpa armigera, Beauveria, Fusarium, Trichoderma, Alternaria, Mucor, Epicoccum*

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Its done!!!

This has been an incredible steep learning curve, one which I have thoroughly enjoyed. I hope to continue and be able to add to this knowledge.

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Chapter 1

Introduction

1.1 Maize in agriculture

Maize (*Zea mays*, Poaceae) has been domesticated from teosinte-a wild grass, approximately 7-10,000 years ago and is thought to have originated from Mexico. The Oxford dictionary describes the word 'maize' as being derived from *mahiz*-Taino and *maíz*-Spanish around the mid-16th Century. The term maize and corn today are interchangeable and generally have the same meaning. The exception relates to the geographical location the crop is grown. For example in Scotland and Ireland corn means oats, in England corn means any cereal crop including wheat, while in the USA and Canada corn and maize mean the same.

The term maize today is specific to *Z. mays* but applies to the whole plant; grain (kernel), stem, leaf and roots. *Z. mays* is more commonly referred to as maize in the scientific and farming sectors (dairy sector) in NZ, especially when the plant is used for other commercial agricultural products. The word corn is more often associated with food products (human consumption), especially when the grains are used in cereals as in popcorn. For this thesis the term maize will be used and applies to all growing parts of the plant.

Worldwide maize is an important food crop for both humans and other animals. In NZ maize is grown for both silage and grain with the end products primarily for stock food (58%) and the remainder for human consumption and the industrial processing sector (42%) (Booker 2009). The popularity of maize as a supplementary stock food for the dairy industry has grown rapidly in NZ (Booker 2009; Millner and Roskruge 2013). Maize grain and silage have a higher food value than more traditional feed like grass-produced silage or hay with a good cost to dollar/kilogram return (Densley *et al*. 2003). When making silage, the whole maize plant is shredded and packed into a large stack (bun) and stored anaerobically until winter feeding. For grain, the maize seed is harvested off the kernel or cob, stored in silos and is later milled for poultry, pigs and cattle including dairy cows.

Dairy farming is an economically important industry in NZ, providing 37% of total primary industry export value, which equates to $13.2 billion to the economy (Dairy NZ-Quick stats http://www.dairynz.co.nz/media/3142896/QuickStats-about-dairying-new-zealand.pdf). NZ provides 3% of the world's milk products and the industry employs over 48,000 people. Given the importance of dairy stock to the economy, selected strains of maize are being developed to enhance the feed quality by DuPont Pioneer® and Advanta seeds (previously known as Pacific seeds), specifically suited for the environmental conditions of a given geographical region. These strains are bred to

enhance the plant's ability to withstand disease, insect and/or climate pressures while retaining good production such as growth and biomass.

1.2 Important pathogens affecting maize in NZ

There are numerous fungal diseases of maize however only a few diseases commonly occur in NZ. The diseases covered in this thesis include; 1-*Setosphaeria turcica* (formerly known as *Helminthosporium turcicum*) causing the disease Northern leaf blight (NLB) also known as Northern corn leaf blight, 2-*Aureobasidium zeae* (Eyespot) and 3-Diseases caused by *Fusarium* spp.

1-*Setosphaeria turcica*

S. turcica causes the disease NLB in maize and is widespread throughout NZ (Perkins 1987). The fungus overwinters in crop residue and the spores are spread by the wind, capable of travelling long distances. Symptoms include distinct cigar shaped lesions on the leaf and visible grey shades may be present on the underside of the leaf. Fungicide treatment is not usually an option as there are few fungicides registered for use and by the time the disease is noticed the crop is too tall to spray. The disease NLB decreases the yield and while commercial companies like DuPont Pioneer® continue to develop resistant strains the disease can still infect the plants resulting in decreased yields (Lipps *et al*. 2004).

Figure 1.1 NLB damage in a maize crop. Photo courtesy of DuPont Pioneer®. https://www.pioneer.com/home/site/us/agronomy/cropmanagement/corn-insect-disease/northern-leaf-blight/

2-*Aureobasidium zeae*

Aureobasidium zeae causes a disease called Eyespot that is spread by wind and moisture (Arny *et al.* 1971; Munkvold and Martinson 2001). It survives overwintering in crop residue with spores produced on the underside of the leaves (Lipps and Mills 2005). The disease attacks the maize leaf sheaths and the leaves covering the ears, causing leaf necrosis and interfering with leaf photosynthesis resulting in reduced growth, reduced yields and dieback (Arny *et al.* 1971; Lipps and Mills 2005).

Figure 1.2 Eyespot in maize. Photo courtesy of DuPont Pioneer®. https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/eyespot/

3-*Fusarium* spp.

Fusarium spp. affects the quality and yield of maize (Czembor *et al*. 2015). Species of *Fusarium*, such as *F. verticillioides* and *F. proliferatum,* produce mycotoxins, which are secondary metabolites. The main mycotoxins are deoxynivalenol (DON), zearalenone (ZON) and fumonisins which can affect stock health (Czembor *et al.* 2015; Fink-Gremmels 2008). The mycotoxins can cause health problems if consumed by humans and livestock varying from minor upsets through to life threatening conditions, e.g. liver cancer in humans (Czembor *et al.* 2015). The extent of the contamination may mean the plant and/or crop may have to be discarded and is no longer suitable as stock food (Fink-Gremmels 2008).

Figure 1.3 *Fusarium* ear rot causes damage to the corn ear and produces mycotoxins. Photo courtesy of DuPont Pioneer®. https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/corn-ear-rots/

There are numerous species of *Fusarium* responsible for several diseases in maize targeting the stalk, root, ear or kernels, e.g. *Fusarium* ear rot is visible on the tip of the ear or cob (Fig. 1.3) and *Fusarium* stalk rot (Fig. 1.4). *Fusarium graminearum* (telemorph-*Giberella zea*) can also result in seed rot, seedling blight, root rot and ear rot (Asran and Buchenauer 2003). *Fusarium moniliforme, F. verticillioides* and *F. proliferatum* can all cause seedling diseases such as seedling blight and seed rot, root and crown rot, stalk and ear rot (Munkvold 2003; Nelson 1992). More than ten *Fusarium* spp. cause seedling blight, wilts, seed and root rots on maize such as *F. oxysporum, F. equisetti, F. culmorum, F. acuminatum, F. graminearum* (Asran and Buchenauer 2002; Leslie *et al.* 2008; Munkvold 2003).

There are two main diseases affecting the ear and kernels (Munkvold 2003). Giberella stalk rot is usually caused by *F. graminearum* but *Fusarium culmorum* can also be responsible (Czembor *et al.* 2015). The kernels display a distinct pink colour from the tip towards the base and may encompass a large area of the ear (Munkvold 2003). The other disease is Fusarium ear rot (Fig. 1.3) caused by several *Fusarium* spp.; *F. verticillioides* (syn. *F. moniliforme*), *F. proliferatum* and *F. subglutinans*, all produce identical symptoms with light pink mould growing on the kernels.

Figure 1.4 *Fusarium* stalk rot breaks down the stem causing death of the plant. Photo courtesy of DuPont Pioneer®. https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/fusarium-stalk-rot/

1.3 Insect pests of NZ maize

There are four main insect pests of maize in NZ; 1-Argentine stem weevil (ASW) (*Listronous bonariensis*) (Coleoptera), 2-Greasy cutworm (*Agrotis ipsilon*) (Lepidoptera), 3-Corn earworm (*Helicoverpa armigera*) (Lepidoptera) and 4-Grass grub (*Costelytra zealandica*) (Coleoptera).

1-ASW is commonly found throughout NZ (PestWeb 2014; Watson 1981). The adults feed externally on plant leaves; lay their eggs which hatch and the subsequent larvae then burrow toward the base of the stem targeting the growing point of the plant (Watson and Hill 1984). The larval stage has the most economic impact with only one larva at the leaf mining stage capable of killing up to four plants by eating the centre or growing point of the tiller (Fig. 1.5). Treatment is difficult as spraying the insects is ineffective at the larval leaf miner stage as chemicals cannot penetrate the leaf surface. The rate of sowing seeds is denser in forage crops than for seed crops. This makes it easier for pests to travel between plants in forage crops. To date control has been through seed treatment and pasture management. The previous season's plant debris must be removed before planting but this still does not offer full protection (OEPP/EPPO 1989; PestWeb 2014; Watson and Hill 1985; Watson 1981). Allowing the sun to dry the crop residue between tractor workings is also a suggested as a method of cultural control.

Figure 1.5 Argentine Stem Weevil damage to a maize crop. Photo courtesy of DuPont Pioneer®. http://www.pioneer.co.nz/news/2016-08-30/new-research-highlights-seed-treatment-returns.html

2-The greasy cutworm adult is a nocturnal moth found throughout NZ but the population tends to fluctuate in densities and may not be seen in one season but prolific in the next (Addison 2007). In epidemic outbursts the peak time of damage is October through to April and severe damage can wipe out entire rows of maize. From the 1st to 3rd instar stages the caterpillars feed on the leaves (Watson 1981). By the 3rd and 4th instar stages, where the larvae can cause the most damage, the lava may burrow inside the plant then sever the whole plant off at ground level (Fig. 1.6). This makes it hard for farmers to determine actual levels of infestation in the crop. One caterpillar alone can destroy large areas in young crops with, in warmer climates, up to three generations completed in a year. (Addison 2007; Watson 1981). The height of maize plants can make chemical control difficult, especially when combined with the larval behaviour burrowing underground therefore making application of pesticides ineffective.

The ASW and greasy cutworm survival into the next season is dependent on overwintering in crop debris (PestWeb 2014; Watson 1981). Cultural control methods such as reduction of debris left in paddocks and paddock rotations can be used as a management tool to limit pest numbers but these methods do not eradicate the pests from pastures.

Figure 1.6 Greasy cut worm damage seen as plant severed and lying flat on the ground. Photo courtesy of North Carolina State University, https://entomology.ces.ncsu.edu/field-corn-insects/scouting-and-thresholds/scouting-for-seedling-insects/

3-The corn earworm is a polyphagous agricultural pest and is common throughout the world. It is attracted to the fruiting parts of a wide range of crops but prefers maize when available (Fefelova and Frolov 2008). Eggs are laid on the upper leaf blade or silk, the caterpillar then travels to the corn ear and kernel (preferably when at the milk-wax stage of the seed) to feed however it will also eat the leaves. The corn earworm will normally complete an average of six instars before pupation into an adult moth. Control of the corn earworm is difficult as the larvae are protected inside the kernel and resistance has developed to some chemical sprays (Cameron and Walker 2004). Chemical pesticides are expensive and the crop height of maize limits spraying, especially when the ear develops. The ear development is usually close to harvest time and spraying may not always be permitted as the chemical residue may also remain on the crop. The end use of the maize, i.e. whether for human or animal consumption, will determine if application is acceptable.

4-The grass grub is a native beetle of NZ and a pest of pastures and crops (Young *et al.* 2009). While the adult will eat leaves, the majority of the damage done to maize is caused by the larvae (Jackson *et al*. 1990; Townsend 2002; Young *et al*. 2009). The larvae consume the plant roots, causing the death of the plant, then move on to the next plant. The larvae will eat the roots from seedling stage right through to mature plants (Cliffe 2011; Jackson 1990; Young *et al*. 2009). The larvae have three instar stages with the 2nd and 3rd instar causing the most damage, occurring between March and July (Cliffe 2011; Jackson 1990; Townsend 2002). The larvae will develop either in a one or a two year cycle depending on climatic conditions. The 2nd and 3rd instar are found in the top 25 mm of soil. The

larvae move closer to the surface by the 3rd instar and will be within the top 2 cm by late summer-early winter (Jackson 1990; Young *et al.* 2009). Potentially large areas of pasture are affected depending on the density of grubs. Damage is seen as bare patches in pastures and crops, some weeks after, making it more difficult to control and prevent.

Control can be difficult, depending on the larval instar stage, as the chemical pesticide does not penetrate deep enough into the soils (Cliffe 2011; Townsend 2002). Cultural control methods include cultivation of affected paddocks by ploughing, heavy rolling or stock tramping, or increasing the number of stock in the mob. These methods can all help reduce numbers but in severe infestations insecticide sprays may be needed. Insecticides will only be effective when the larvae are towards the soil surface (Cliffe 2011; Jackson 1990; Townsend 2002). Seeds can be coated with insecticides to prevent damage while the seedlings establish but this is effective only short-term lasting just one season. Trials with the bacterium *Serratia entomophila* as a Biological Control Agent (BCA) had limited success (Jackson 1990; Popay *et al*. 2003; Young *et al.* 2009). Jackson (1990) suggested the addition of *S. entomophila* used as a seed coating should be used in conjunction with cultural practises such as grazing management and using grass resistant seed strains. Young *et al*. (2009) used a seed coating technique of two entomopathogenic bacteria, *S. entomophila* and *Yersinia* sp. with the addition of *S. entomophila* dramatically increasing the seedling establishment of wheat in NZ. Farrell and Stufkens (1977) noticed while maize was attacked by the grass grub it was to a lesser degree than grasses and clovers and the grubs themselves did not have the same growth rates. A different approach by Popay *et al*. (2003) was with the use of a fungal endophyte in meadow fescue (*Fescue pratensis*). They trialled the fescue with and without the endophyte, *Neotyphodium uncinatum,* and measured for bioactivity by larvae weight of grass grub. They found with fescue plants containing endophytes had fewer roots eaten and the larvae had lost weight. This approach suggests a beneficial endophyte added to a different crop such as maize may be useful for a pest and disease management approach.

1.4 Endophytes

The term endophyte literally means inside the plant (Greek for *endon* -inside and *phyton* -plant) and has been used broadly to include a wide variety of lifestyle traits (Schulz and Boyle 2005). An endophyte can be a bacterium or fungus and live within the plant tissues without causing harm or disease and in fact may give a benefit to the host (Rodriguez *et al*. 2009; Zakaria *et al.* 2010). Endophytes can occur in both above and below ground plant tissues often forming a mutualistic relationship with plants where the fungus gains protection from the environment with freely available nutrients from the plant (Hardoim *et al.* 2015; Saikkonen *et al.* 1998). The plant in return

may receive benefits such as enhanced protection from pests and diseases and/or there may be an increase in stress resistance (Araujo *et al.* 2000; Raman *et al*. 2012; Rodriquez *et al.* 2009; Zakaria *et al.* 2012). There is debate over the use of the term 'endophyte.' For example mycorrhizal fungi can be beneficial and can live both externally and internally but are usually not referred to as endophytes (Hardoim *et al.* 2015; Hyde and Soytong 2008). Whereas ectomycorrhizal fungi are fungi that live outside and grow into the rhizosphere but can also colonise within the plant roots but these have been referred to as endophytes by Rodriguez *et al*. (2009). The term 'endophyte' may also include different lifestyle traits such as the fungus being a latent pathogen or as part of the reproductive life cycle the fungus may exit the plant, especially on senescence, to reproduce and sporulate (Hardoim *et al.* 2015; Hyde and Soytong 2008; Stone *et al.* 2017).

In the review by Rodriquez *et al.* (2009) endophytic fungi were categorised according to taxonomy: clavicipitaceous (C) containing class 1 endophytes and nonclavicipitaceous (NC) containing classes 2-4. The criteria for each class was characterised by the tissues colonised, the amount of colonisation within the tissues, transmission-horizontal or vertical, and the host range.

The C group of endophytes include the grass endophytes which have been well studied, such as *Epichloë* spp. (anamorph = *Neotyphodium*). These endophytes are naturally occurring in grasses including ryegrass (*Lolium perenne*). The endophytes produce alkaloid toxins, lolitrem B and ergovaline, causing conditions such as ryegrass staggers and heat stress respectively. Other alkaloids (loline and peramine) produced by the endophytes have more of a deterrent effect on insects than on grazing stock (Popay *et al.* 2012). These endophytes, such as *Neotyphodium lolii* used in the development of AR37 by AgResearch NZ, have been selected that show minimum toxicity to sheep, cattle, dairy cows and horses (Hume *et al*. 2004; Popay *et al.* 2012). Since the development of inoculating endophytes into grasses, specific endophytes have been selected to deter or reduce insect population specific to the target pasture pests. For example an endophyte (AR37) which produces more peramine than lolitrem B can deter attack and feeding from ASW, Porina (*Wiseana* spp.) and root aphids (*Aploneura lentisci*) in perennial ryegrass and also potentially increasing the plants fitness (Charlton and Stewart 1999; Clay 1989; Popay and Hume 2011; Popay *et al.* 2012).

While the NC fungi are much less studied they are a more diverse group. Both above and below ground colonisation of plant tissues can occur. The NC group are further divided into three functional classes; life history, ecological interaction and other traits (Rodriquez *et al.* 2009). Classification of the fungi into classes is by traits such as; host range based on which tissues have been colonised, through the type of reproductive structures, whether the fungus is transmitted vertically and/or horizontally between plants, or whether the species diversity is low or high (Rodriquez *et al*. 2009; Vidal and Jaber 2015). By dividing fungal endophytes into groups a better understanding of the

fungus-plant interaction can be gained and this knowledge may then be applied to the selection of endophytes specific to pest and/or diseases in chosen plant species. Some examples like *Beauveria bassiana, Lecanicillium lecanii* and *Metarhizium anisopliae* are already available as commercial products to control insects by direct chemical applications. The previous fungi are known as entomopathogenic fungi. As the name suggests they are pathogenic to insects but it has recently been shown they are also capable of endophytic colonisation (Vidal and Jaber 2015). This is an important area as both *B. bassiana* and *M. anisopliae,* among others, have been and are continuing to be developed for biocontrol agents (BCA) for agriculture crops.

It has been suggested by Pan and May (2009) that the fungal endophytic community is a well-structured assembly of different fungi which differ between internal sections of the plant such as the leaves, stem and the roots. Some endophytes are known to colonise different plant tissues (Hardoim *et al.* 2015). This is referred to as multiple habitat levels (Pan and May 2009). For example, *Trichoderma* spp. colonise roots while *Alternaria* spp. have been reported to colonise the stems or leaves. Others such as *Epicoccum* and *Fusarium* species have been found in seeds from fresh cobs of maize as well as other areas of the plant (Fisher *et al.* 1992). Rodriquez *et al.* (2009) note the leaves in tropical forests contain numerous independent infections and are of high diversity.

Colonisation by endophytes is variable and depends on the host plant, environmental conditions and fungal species and strain (Carrol 1988: Hardoim *et al.* 2015; Rodriguez *et al*. 2009). Vidal and Jaber (2015) suggested the fungal isolate-host relationship is extremely important to have the right combination for the endophyte to establish in the host plant. It is suggested that even the strain of the fungus and the soil mix has an influence on success of endophytic colonisation. The method of colonisation is just as variable, occurring by entry into the roots, stems or leaves or by vertical transmission or horizontal transfer (Bais *et al.* 2006; Hardoim *et al*. 2015; Rodriguez *et al*. 2009; Stone *et al.* 2017). The endophyte may travel systemically through the plant colonising different tissue types but where it colonises depends on the specific endophyte and the species of host plant (Hardoim *et al*. 2015; Rodriguez *et al.* 2009).

1.4.1 Maize endophytes

Endophytes are known to occur in maize but there is little information on the diversity of naturally occurring endophytic species, which plant tissues they colonize or whether they offer the plant protection from pests and disease (Araújo *et al.* 2000; Vidal and Jaber 2015). Furthermore, it is unknown if there is any correlation of endophyte genotypes with geographical location between Waikato and Canterbury.

The stem and leaf flag of maize were the focus of a study by Fisher *et al*. (1992) in UK, which looked at both bacterial and fungal endophytic communities without any visible sign or symptoms of disease. They found that when the fungal diversity was low there was a high bacterial diversity present in the cobs of maize plants. Fisher *et al*. (1992) also examined the vertical transmission and tissue specificity of certain fungal and bacterial populations. Healthy plants were selected to test if populations of fungi and bacteria co-exist in maize. Their results suggest the distribution patterns of these organisms were different between the lower plant, core or pith of the stem and the leaf and tip areas of the plants studied. They noted that the majority of the bacterial and fungal species show a high degree of tissue specificity. For example bacteria were found in the core of the stem closer to the ground while more fungal colonies were recovered towards the lower and middle parts of the stem. An example was *Alternaria alternata* which was associated exclusively with the leaves. Pan and May (2009) studied the internal plant habitat of fungal communities and tested the lower leaf, ear stalk and upper leaf for whole communities in maize, using both culture dependent and culture independent methods for interspecific common patterns as well as community assembly. They found that interspecific interactions affect the endophyte community species composition but this is influenced by the host's habitat as well. The fungi found in the previous study contain multiple life history traits of pathogens, latent pathogens, saprophytes and endophytes. Determining which state each fungi has presented depends on the site found (internal or external of the plant as well as leaves or roots) and the time each are found (i.e. close to senescence or if the plant was diseased or not) (Carroll 1998; Fisher *et al*. 1992; Schultz and Boyle 2005).

An interesting study by Darvas *et al*. (2011) looked at the interaction of the caterpillar *Helicoverpa armigera* and the disease causing fungus *Fusarium verticillioides* in genetically modified maize. They noticed the insect did not grow beyond the third larval instar stage and it would try to move away from the fungus in the cobs. They also noted that, while the larvae did transfer the fungus to other places, there was no development of maize pink ear rot disease.

Roots and kernels were examined for endophytes in the study by Seghers *et al*. (2003). This study looked at how agricultural practises, such as agrochemical use, can influence endophytic communities of both bacteria and fungi groups. The authors used plating techniques and counted colony forming units (CFU's) to detect communities. DNA was taken from soil and plant (roots and kernels) samples, followed by denaturing gradient gel electrophoresis (DGGE) to fingerprint the endophytic community for comparisons between the two groups. The overall results showed agricultural practises do influence certain populations of the root endophytic communities. The highest diversity was found in soils with natural organic fertilisers applied while the soils with chemical herbicides applied were low in diversity.

Rodriquez *et al*. (2009) reviewed numerous studies and concluded that endophytes can increase the plant fitness to abiotic and biotic stresses. The plant's fitness to cope with insects and disease is more tolerant if endophytes are present. The review also concluded that endophytes can be specific to host plant species and the tissue colonised. A study by Singh *et al*. (2011) also adds to the evidence of endophytes contributing to the plant's ability to cope with stress. Their study looked at both class 1 and class 2 endophytes with stress tolerance. They looked at the plants' ability to cope with drought, heat, water stress and salinity in conjunction with the mechanisms involved in that response with different endophytes and hosts plant species. They concluded the effect varied depending on host species and which endophyte was present, along with which stress was applied.

The studies mentioned above did not sample the 'whole' plant but concentrate on specific areas. It is unknown if these endophytes are the same throughout the plant. For the present study, the plant was sampled over the entire length of the plant and repeated for each plant sampled. It is hoped a more conclusive overall picture from the whole plant can be determined from these results and determine if species are throughout the plant or specific to certain areas.

1.4.2 Endophytes and disease

A study by Zakaria *et al*. (2010) found 110 fungal isolates from the rice paddy plant (*Oryzae sativa*) with the isolates being found from all plant habitat levels. The study looked at healthy plants with no obvious disease symptoms and to isolate the naturally occurring fungi. Interactions between endophytes and host plant may change from mutualistic to pathogenic depending on stress factors or vice versa hence the reason for selecting healthy plants. Fisher *et al*. (1992) and Schultz and Boyle (2005) have also cited latent pathogens, found in their studies, becoming pathogenic given the right conditions such as *Fusarium*, *Curvularia, Penicillium* and *Aspergillus*. Zakaria *et al.* (2012) suggested that the latent pathogens found in plants, if put under stress, may become pathogenic. It is therefore possible that latent pathogens may be found to be naturally occurring in maize plants and would be hard to distinguish from endophytes without further investigation (Fisher *et al*. 1992; Zakaria *et al*. 2010).

The discovery of the endophytes in grasses led to the isolation and selection of strains which produce higher levels of alkaloids, (secondary metabolites produced by the endophytes), in response to herbivory (Popay and Hume 2011). One main endophyte genus concerned is *Epichloë* spp. but the name '*Epichloë*' now generally refers to both sexual states (Clay 1989; Kuldau and Bacon 2008). Inoculating endophytes into grasses can result in the development of specific mutualistic associations such as; drought tolerance, resistance to vertebrate and invertebrate pests and fungal diseases

(Kuldau and Bacon 2008). Endophytes are commonly associated with agriculture pasture grasses (Poaceae) such as ryegrass (*Lolium perenne*) and fescue species (*Festuca* spp.). The potential for development of these endophytes in new host combinations can only add and benefit agriculture (Easton and Fletcher 2007).

1.4.3 Endophytes and insects

As mentioned above, some endophytes are also referred to as entomopathogenic fungi (Guesmi-Jouini *et al*. 2014; Vidal and Jaber 2015). A well-known and studied example is *B. bassiana* that has the ability to inhibit plant pathogens as well as insect development (Ownley *et al*. 2004; Wagner and Lewis 2000). The addition of an entomopathogenic fungus within a plant has the potential to control a number of pests. Numerous insects have been studied with the use of entomopathogenic fungi in different plants and crucially, the plant remains unaffected (Bing and Lewis 1993; Bruck 2010; Ownley *et al.* 2004). One such study applied *B. bassiana* by spraying directly onto maize plants and found the application to be effective at controlling European corn borer (*Ostrinia nubilalis*) (Ownley *et al*. 2004). The authors also noted *B. bassiana* was found later to be endophytic in the plant. *Bionectria ochroleuca* and *B. bassiana* have been found as endophytes in artichokes (*Cynara scolymus)* and known to have high virulence on the artichoke aphid (*Capitophorus elaeagni*) (Hemiptera: Aphididae) (Guesmi-Jouini *et al.* 2014; Raman *et al*. 2012). While this study showed the endophytic capabilities of the fungi it does not mention any effect against insects when the fungus was added as an endophyte to the plant.

Therefore selection of an endophyte for a BCA will depend on the effect and targeted insect as well as the fungal strain and host plant compatibility. The chosen endophyte must be capable of endophytic colonisation in the host plant.

1.5 Research objectives

Little is known of the endophytic community of the entire maize plant rather studies focusing on particular areas. In this study, putative endophytes were isolated from within the plant tissues from roots to the tip of the plant. For the purpose of this study the term endophyte refers to any fungus found within the plant after surface sterilisation with no visible disease symptoms on the plant. This could include a latent pathogen as well as beneficial endophyte however the latent pathogen can exist in a plant asymptomatically until a trigger ‘switches on’ the fungus to become disease causing (Hardoim *et al.* 2015; Hyde and Soytong 2008). For this thesis, the lifestyle trait is not the purpose of

the study but more important is the location as coming from 'within' the plant. This makes the surface sterilising methods (Chapter 2) extremely important to remove the possibility of anything other than the isolate being an endophyte. Out of interest, each fungus identified has been reviewed for the possibility of more than one lifestyle trait (Appendix D1).

In this study it was looked at what part of the plant the endophyte colonised and if there was a difference between fungal groups. Two geographical areas (Canterbury and Waikato), NZ, were tested to determine if areas differed in species composition with five sites per area being tested.

Fungi were identified by DNA extraction then sequenced and confirmed where possible by morphological traits to species level. The sequenced results given from BLAST and UNITE were above 98% and a consensus from the both sites. A further aim of the project was to determine if any of the identified fungi have potential to act as a BCA. The aim was to find a naturally occurring endophyte or endophytes that could deter or kill insects and/or prevent disease. The isolate could be taken up through the seed coating to become endophytic in the plant. This could greatly enhance farmer's reliance on chemical sprays (less acceptable in today's environment) and could be an environmentally friendly pest management tool (Ownley *et al.* 2004; Seghers *et al*. 2004). It was mentioned earlier (Pests section-pg. 7) that *H. armigera* have a preference for maize plants (Fefelova and Frolov 2008). For this reason and ease of supply, *H. armigera* was used for bioassays. The pathogen *S. turcica* was selected to test against plants inoculated with potential endophytes. This disease has been used previously in another study from our Laboratory with some good results. Samples of infected plants were tested for evidence of endophytic presence in each insect and disease trials.

It has been known endophytes are in seeds with a study by Fisher *et al.* (1992) finding 68% of seeds used for planting their crop contained endophytes. However young seeds obtained from the mature cobs only returned 7%. The seed supplied by DuPont Pioneer® were tested to check for the baseline resident endophyte community population.

Chapter 2

General methods

2.1 Introduction

In this section the methods are given for the processing of the plants (2.2) through to identification (2.2.4), and then application and testing of isolates against plant pathogens (2.4) and insect pests (2.5). Methods are also provided for the screening for background endophytes (2.6) on the seed resident endophyte community supplied by DuPont Pioneer®.

2.2 Sampling field collected maize plants for endophytes

Screening of NZ maize plants for endophytes was done over two seasons (2014 and 2015 summer seasons). Two regions, Waikato and Canterbury, were selected for screening to identify endophytes naturally occurring within maize plants. Through the Foundation of Arable Research (FAR), maize crops were sampled, by taking whole plants and processing for isolation of fungi present (Table 2.1). Plants collected by FAR from the North Island (Waikato) were sent by courier while the Canterbury plants were collected by myself. Five plants per site, with plants coming from six different farms from two geographical regions per season (November to May) were collected, for a total of 22 plants in 2014 and 12 plants in 2015. The processing time, by one person, before a plant showed visible saprophytic growth was less than one week, even with cold storage. This limited the number of plants that could realistically be sampled at one time within a season.

2.2.1 Recovery of putative endophytes from maize

Maize plants were divided into sections (Fig. 2.1); 1-roots, 2-ear, 3-upper stem (which included the flower if present) and 4-lower stem. Each section was further subdivided. Roots were divided into three sections; seminal, radicle (also called lateral roots) (Fig. 2.2a) and brace (adventitious roots) (Fig. 2.2b). The radicle roots contain fine hairs and are found spread throughout the root system (Fig. 2.2a). Samples were taken at random for radicle and seminal root samples. The samples for the brace roots were taken from the brace node (Fig. 2.2b), as close as possible to the stem just above the ground, extending to below the soil surface with sufficient length to give multiple tissues samples for plating .

A selection of root samples were taken at random and plated onto agar as a group containing 2-4 roots (Fig. 2.3) with five tissue samples arranged per plate. The three types of root samples were kept separate with two replicate plates for each root type giving a total of six plates, thirty root tissue samples in total, sampled from one plant.

The ear was divided into four sites with enough tissue sample taken and plated on agar from each site. Each of the four sites from the ear (Fig. 2.1-2a-d) had five tissue samples arranged per plate (Fig. 2.4a) and two replicate plates were tested per plant giving a total of 40 tissue samples. The leaf husk (Fig. 2.1-2a), a group sample of silk (Fig. 2.1-2c) consisting of approximately ten hair thickness, kernels (Fig. 2.1-2b) and a section of the peduncle (Fig. 2.1-2d) cut in half length wise depending on the thickness to fit inside a Petri dish were all plated on agar.

Table 2.1 Origin of maize plants for sampling for 2014 and 2015 seasons.

Site	**Region**	**Variety of seed used**	**Date planted**	**Sample plant number**
2014				
1	Waikato	Pioneer P0021	25/10/2013	1-5
2	Waikato	Pioneer 34N41, Waxy hybrid	10/10/2013	6
3	Canterbury, Ashburton	38V12	9/10/2013	7-11
4	Waikato	P0021 hybrid	8/10/2013	12-13
5	Canterbury, Tram Road	39T45	15/10/2013	14-17
		38v12	25/10/2013	18-20
6	Canterbury, Oxford	Super sweet NZ yellow 55630, F1 hybrid	NA	21-22
2015				
1	Waikato	P0021	13/10/2014	1-5
2	Canterbury, PGG Wrightsons Farm, Lincoln	NA	NA	6-10
3	Canterbury, Lincoln University (LU)	Corson	15/12//2014	11-12

NA- not available

The lower (Fig. 2.1-4a-c) and upper stems (Fig. 2.1-3a-d) contained top and bottom leaf samples from each section taken close to the stem as well as a section of stem tissue again cut in half to be thin enough to fit within a Petri dish. The upper stem region (Fig. 2.1-3a-d) had four areas sampled from the very tip of the plant testing the tassel (Fig. 2.1-3b), then the top leaf (Fig. 2.1-3a), a section of stem (Fig. 2.1-3c) and a lower leaf (Fig. 2.1-3d) in this area. Five tissue samples per agar plate with two replicate plates giving a total of 40 tissue samples taken from this area. The lower stem area (Fig.

2.1-4a-c) had three tissue samples taken from the top leaf (Fig. 2.1-4a) inner leaf (Fig. 2.1-4b) and the stem (Fig. 2.1-4c), again five tissue sample per plate (Fig. 2.4.b), with two replicate plates on agar giving a total of 30 tissues samples from this region. The same process was repeated for each plant making sure the same area was sampled for every plant from each geographical region in each season.

2.2.2 Surface sterilisation of maize tissues

The term surface sterilisation for this study refers to all external microbes killed or removed from the exterior of the maize plant tissue. The internal tissues remain unharmed allowing the endophyte to exit when plated on potato dextrose agar (PDA) (Difco, NJ). Firstly all soil residues were rinsed off with tap water and air dried. Each tissue sample was processed following the protocol below. For surface sterilisation, samples were sequential placed in a deep Petri dish containing: 0.01% Triton X-100 (BDH) solution for three minutes, agitated gently 2-3 times, removed and placed into the next Petri dish containing 2% of sodium hypochlorite (bleach; Cyclone-Diversey) for five minutes, then into 70% EtOH (ethanol) for one minute followed lastly by three rinses in sterile water (dH20) for one minute each. Samples were placed on sterile paper towel to soak up residual water and allowed to air dry in laminar flow for 2-3 minutes before cutting and plating. The plant tissue samples were gently mixed in the solutions to ensure samples were completely submerged. Solutions were changed after 3-4 samples with all solutions replaced between each plant.

After surface sterilisation, the plant tissue samples were cut into approximately two-three centimetre segments with the sterile ends cut off and discarded. Five pieces were placed on PDA after surface sterilisation (Fig. 2.4a & b). More tissue was surface sterilised than needed for plating so tissue samples were taken at random and placed on the plates without coming into contact with neighbouring tissue samples. Plates were incubated at 25°C @ 16 hour light, 8 hour dark cycle for 3-4 days or until visible fungal growth was present (Fig 2.4b).

Control plates: A sample of the 0.01% Triton X-100 solution was plated after washing the tissues to establish the fungal community present in each plant before they had been sterilised. This was considered the positive control. Three plates were taken at random from the water used to rinse the plant tissue for fungi and bacteria presence. These plates were expected to have no bacteria or fungal colonies hence these were called the negative control. Checking was done by pipetting 100 µl of the rinse water and with a cell spreader (Biologix) spread onto a PDA plate. Two other controls tested the sterility of the chopping board and the external plant tissue after surface sterilisation. This was tested by applying 200 µl to the sterile chopping board, gently rubbing then collecting 100 µl and plated on PDA. The sterile plant tissue was gently rubbed and rolled over the PDA plate and this was

compared to an unsterile plant tissue rubbed over a PDA plate. On first use of this method controls were positive with one fungal colony and some bacterial contamination therefore times were increased for Triton X-100 from one to three minutes and bleach changed to five minutes. Protocols were adjusted until plates were clear i.e. no bacterial or fungal growth after 5 days incubation at 25°C.

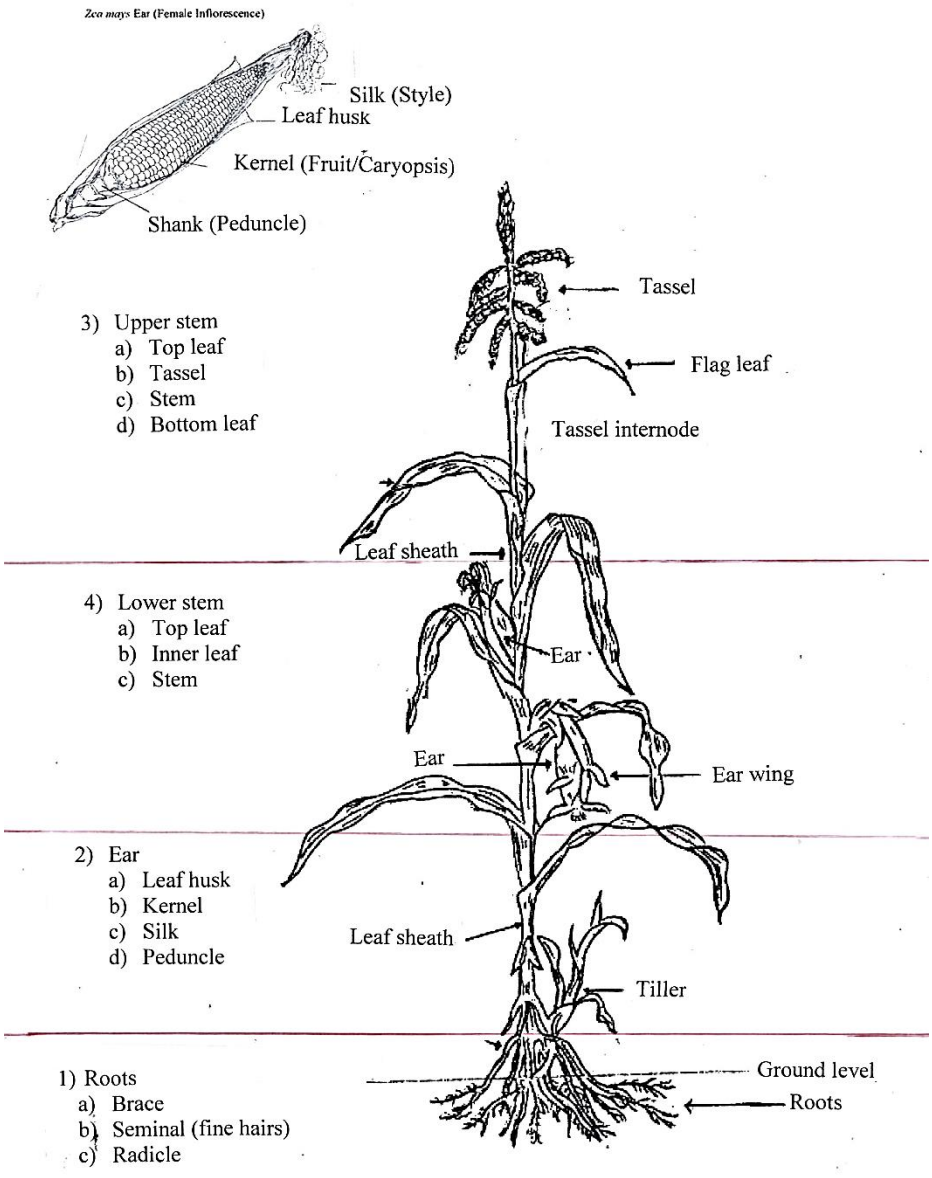

Figure 2.1 A maize plant outlining the sample regions with each region giving the sample sites used for sampling. Photo courtesy of http://www.inspection.gc.ca/plants/seeds/seed-inspection-procedures/field-corn/eng/1347286797332/1347330417322#a45

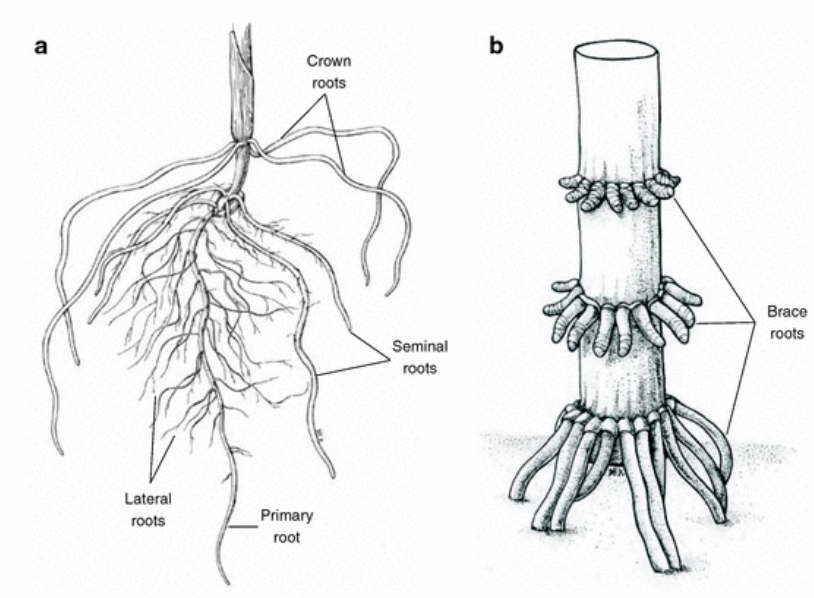

Figure 2.2 Diagram of maize roots; a) showing lateral or radicle roots with fine hairs and the seminal roots- without hairs. b) The brace roots (also known as adventitious or crown roots) may extend from the first or second node to the ground giving the plant another bracing element as it gets taller Diagram from Hochholdinger (2009).

Figure 2.3 Multiple tissue samples per site of roots showing mycelium

2.2.3 Media

Samples of surface sterilised maize sections as prepared above were plated on PDA. It is known that some bacteria interferes or inhibits fungal growth (Fisher *et al*. 1992). This study found it was necessary to add antibiotics to inhibit bacteria (Fig. 2.4b) from some samples. Antibiotics were added to PDA were five hundred mg of chlortetracycline hydrochloride (Sigma) and 2500 mg streptomycin sulphate salt (Sigma) per 100 ml of sterile water. The solution was filtered through a sterile 0.2 µm filter before adding to the PDA. Antibiotics were used at a rate of 1 ml solution to 100 ml agar to give a final concentration of 250 mg/l streptomycin and 50 mg/l chlortetracycline.

2.2.4 Identification of recovered fungi from PDA

Plates were checked daily for fungal growth and the number of morphologically different colonies recorded and axenic cultures produced. For some fungi which were difficult to subculture, potato dextrose broth (PDB; Difco NJ) was used instead of PDA. For these, inoculated flasks, were placed on a shaker at 140 rpm at room temperature (20-22°C) for approximately two days or until mycelium was visible. Gross colony morphology was used to establish different fungal colonies. After colour each isolate had to be considered different in shape, growth or another distinctive pattern, e.g. *Epicoccum nigrum* has a pigment that stained the agar a brown/gold colour. All isolates selected for DNA extraction were deemed to be morphologically different during growth on media or they were from a different plant, plant location, site or the isolate was from a different geographical region.

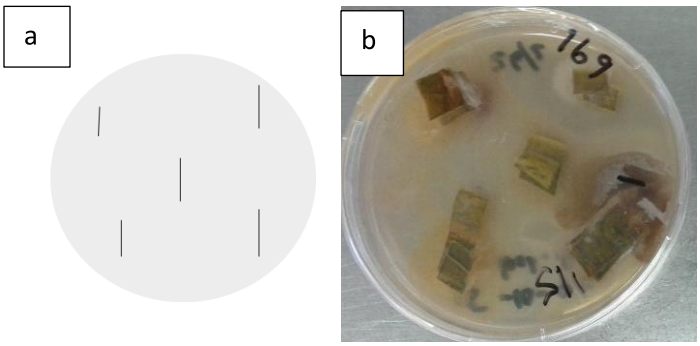

Figure 2.4 a) Arrangement of tissue samples on PDA. b) Leaf tissue arranged on a PDA plate with visible mycelial and bacteria growth.

Microscopy was observed with a Leica DM 2500 light microscope. Slides and cover slips were prepared using a stain mounting medium, Lactophenol blue (LPCB), at half strength. Microscopy was used to look for identifying characteristics such as; hyphae containing septa--presence or absence, spore characteristics or reproductive structures, of species to confirm identification. Visualisation of slides using 20x magnification objective was used for larger macrospores but the smaller spores required the 40x magnification. Identification of the spores and structures were compared with the help of text books; The "*Fusarium* Laboratory Manual" (Leslie *et al.* 2008) was used for identifying *Fusarium* genera to species level and "Genera of Hyphomycetes" (Carmichael *et al.* 1980) and "Illustrated Genera of Imperfect fungi" (Barnett and Hunter 1998) were used for other genera. For example; *Fusarium* species have a range of spore sizes and shapes specific at species level e.g. *F. graminearum* macroconidia spores (Fig. 3.2A) are large and there is no microconidia produced

whereas *F. oxysporum* have microconidia produced on false heads on short phialides formed on hyphae and *F. trinctum* (Fig. 3.9A) produces a distinct tear drop microconidia spore (Leslie *et al.* 2008). Microscopy identified conidiation patterns e.g. *B. bassiana* produces a mass of white 'cotton balls', on a zig zag rachis (spore structure) specific to *B. bassiana*.

Where multiple samples gave morphological identification as the same species from the same plant, only one representative culture was taken for identification. Pure cultures were processed for molecular identification.

2.2.5 DNA extraction

Two to three day old pure fungal cultures were used for DNA extraction. A small amount of hyphae was placed in 500 µl of Chelex 100 Resin buffer (biotechnology grade 5% or 2 g in 40 ml of deionized H_2O). The cells were ground with a sterile pestle then vortexed thoroughly. The tubes were incubated by boiling in water for 12 minutes then cooled to room temperature before centrifuging at 13,000 rpm for 20 minutes. The middle and top clear layer of 100-200 µl was transferred to a new 1.7 ml eppendorf tube, avoiding any cell debris or pellet. DNA was stored at minus 20°C.

2.2.6 Polymerase chain reaction

Two µl of the solution was used for polymerase chain reaction (PCR). Each 25 µl PCR reaction consisted of: 15.75 µl of sterile water, 2.5 µl of buffer (10x) plus $MgCl_2$ (2 mM), 2 µl of deoxynucleotide (dNTP's) (2.5 mM) (Roche), 0.25 µl of Fast start polymerase *Taq* (Roche), 1 µl of each primer (Integrated DNA technologies (IDT)), 0.5 µl bovine serum albumin (BSA, Bio Labs) and 2 µl of extracted DNA per sample. General primers were used which targeted the internal transcribed spacer ITS region of the 16s rDNA using the reverse primer ITS4-5' TCCTCCGCTTATTGATATGC 3'and forward primer ITS5- 5' GGAAGTAAAAGTCGTAACAAGG 3' (White *et al.* 1990). Thermocycling conditions were 95°C for 5 min followed by 40 cycles of 95°C for 45 sec, 57°C for 45 sec, 72°C for 2 min and final extension of 72°C for 7 min. PCR products were visualised on a 1% agarose gel and stained by ethidium bromide or Red safe nucleic acid staining solution for 10 minutes. Samples with single bands present were then sent for sequencing (Lincoln University (LU) Sequencing Unit) and analysed using BLASTN through the NCBI website and UNITE (https://unite.ut.ee/analysis.php) to determine likely species.

2.2.7 DNA analysis

Initially 86 fungi from plant tissue samples were sequenced using both reverse and forward primers (ITS 4 and ITS 5). To keep costs down the remainder were completed with the reverse ITS 4 primer only. In some cases the quality of the reverse sequence was insufficient, therefore a further 14 samples were re-analysed using both primers to get a consensus. Morphology was also used in identification but it was also necessary to have isolates confirmed by sequencing for each plant location (Appendix A. 6) from each plant sampled and for each year.
The top five to eight hits from both UNITE and BLAST were taken to get a consensus. The E-values returned were 0.0 with a query cover greater than 98%. Ideally the isolate identity was greater than 98% but if the identity returned between 92-98% then a consensus of both BLAST and UNITE was used where possible. The unidentified ("unknown") isolates that did not amplify in PCR or gave multiple names at genus level when compared to databases, or BLAST hits of 'uncultured fungus', were marked as unknown. Where possible with unknowns the DNA extraction and PCR were repeated to get an identification.

2.3 *In vitro* bioassay of recovered putative endophytes against a plant pathogenic fungus

Dual culture assays were chosen as a method for screening putative endophytes isolated in the initial screening in maize plants (Appendix A. 1 and A. 2). The maize pathogen, *S. turcica,* causing NLB was chosen to assess the bioactivity of putative endophytes as it is a major disease in maize and can result in up to 30% yield loss in susceptible hybrids (Fowler 1985; Perkins and Pederson 1987). *S. turcica* was found to have varied growth rates between cultures grown on PDA. Previous research also indicated *S. turcica* was slow to form conidia. For this reason an excess number of plates were cultured and over a longer time frame.

For dual culture assays, isolates were sub-cultured onto fresh PDA. Plates were selected with sufficient mycelial growth covering the majority of the plate to obtain enough plugs for the isolates to be tested in each assay. Plugs from both the endophyte isolate and the plant pathogen were placed onto ¼ strength PDA plates (9.75 g PDA + 10 g of bacteriological agar). A template (Fig. 2.5a) was used to mark the placement on every plate for the isolate and the pathogen. Two, 5 mm plugs, were placed opposite each other for both isolate and pathogen (Fig. 2.5b) on each PDA plate with three replicates per treatment. Where the isolate and pathogen were similar in colour the pathogen was marked with a 'P' (Fig. 2.5b). The control plates consisted of 4 plugs on a plate with three replicates.

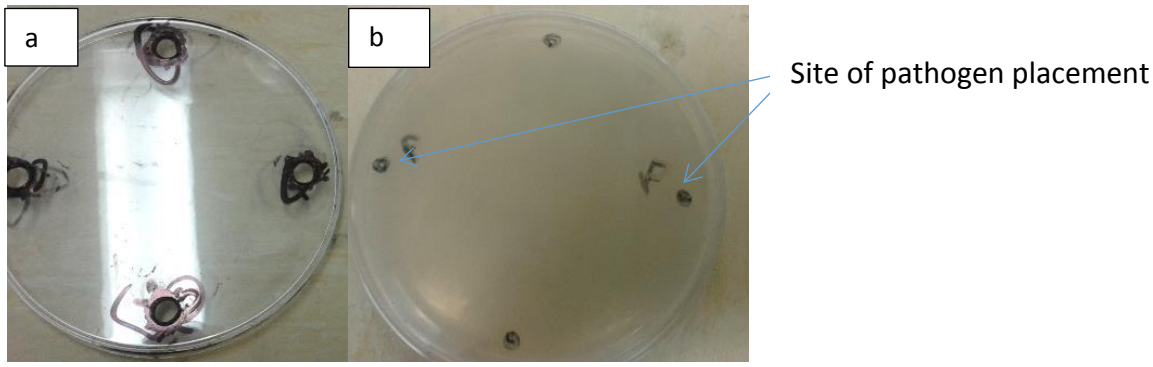

Figure 2.5 a)-The template used for marking the base of PDA plates and b)-placement of pathogen (P).

Dual culture assays were executed in batches of 7-9 isolates tested at a time. All plates were grown at a constant 22˚C. Isolates were grown in the presence of the plant pathogen, *S. turcica,* and growth of the colonies was measured for both pathogen and isolate at seven and 10 days from inoculation. A total of 79 isolates were tested (Appendix B.1). A number of isolates did not survive the initial sub-culturing or storage at 4°C.

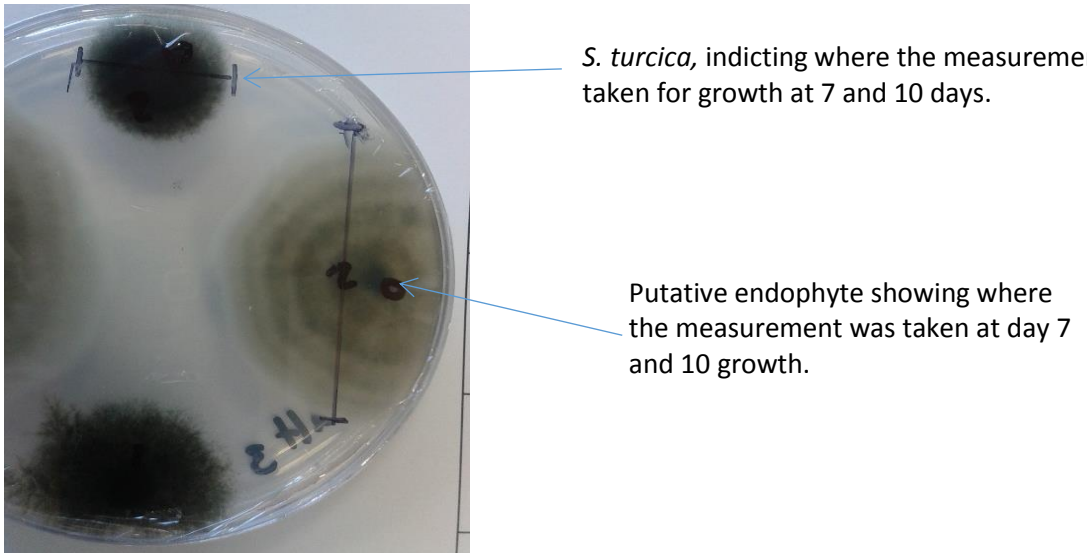

Figure 2.6 The axis of measurements marked across the widest part of each colony, *Alternaria alternata* (12) and plant pathogen *S. turcica.*

Measurements were recorded across the width of each growing isolate colony (Fig. 2.6). A control plate of the pathogen only was grown at the same time. Each isolate was compared to the growth of the pathogen grown on its own (Fig. 2.7).

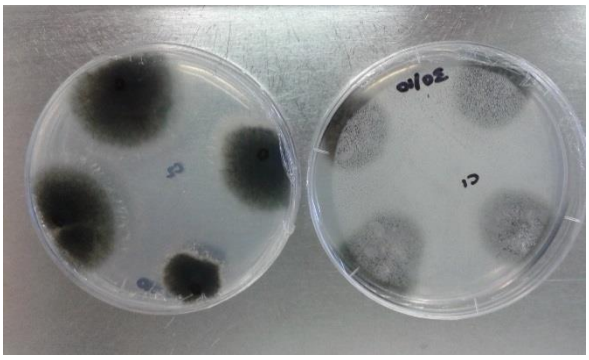

Figure 2.7 *Setosphaeria turcica* control used to compare each isolate (new control used with each assay).

2.4 In planta maize challenge methods

2.4.1 Seed coating

The aim of seed coating was to establish isolates as endophytes and potentially promote plant growth and disease and insect resistance.

Spores were harvested in sterile 0.01% Triton X100 solution with haemocytometer counts of approximately 10^6-10^8 spore/ml to treat maize seeds. The seed coating polymer mix (Flo Rite 3330, BASF) was used, following the protocol of 100 g of seed in 0.56 g polymer + 0.56 g of spore concentration. Equal volumes of 200 µl polymer and 200 µl of spore suspension were mixed and 35 µl pipetted onto the ten seeds. The seeds were gently agitated until all seeds were evenly coated with the polymer-spore solution and container walls were free of solution. The seeds were air dried for approximately 3-5 minutes. For the controls, seeds were coated with a 50:50 mix of 0.01% Triton X and polymer solution. The seeds were placed in the fridge overnight to encourage all seeds to germinate at the same time.

2.4.2 Plant disease assay

To determine the effect of endophytes in disease resistance, the known maize pathogen, *S. turcica,* was selected as a disease challenge. As a result of the dual culture assays, 21 isolates were selected for *in planta* testing (Table 3.4). A preliminary experiment was conducted to ensure that the strain of *S. turcica* was able to generate disease symptoms on these maize varieties. After the preliminary testing it was decided to use both P0021 and 38V12 hybrid seeds supplied by FAR (DuPont Pioneer®) for seed coating disease trials. The DuPont Pioneer® disease score rating indicated the level of effect

of disease with one being highly affected by lesions and necrosis of plant tissue and 10 having no effect. DuPont Pioneer® performance characteristics rated cultivar P0021 at a 7/10 for disease resistance to NLB and 6/10 for cultivar 38V12.

Figure 2.8 Maize plants growing in growth chamber room prior to pathogen application.

After seed coating (method as above) and storage in the fridge overnight, the seeds were removed from fridge and rested, to acclimatise to room temperature before planting. One seed per pot (0.5 litres) was planted using 3-4 month old potting mix supplied by the LU nursery, Springs Road, Lincoln. The pots were placed in a growth chamber at 20-25°C and grown to V4 stage (Fig. 2.9). Prior to the pathogen application plants were kept in separate trays for each seed isolate treatment (Fig. 2.8) until the pathogen was applied. The experiment was conducted five times with different endophyte isolates.

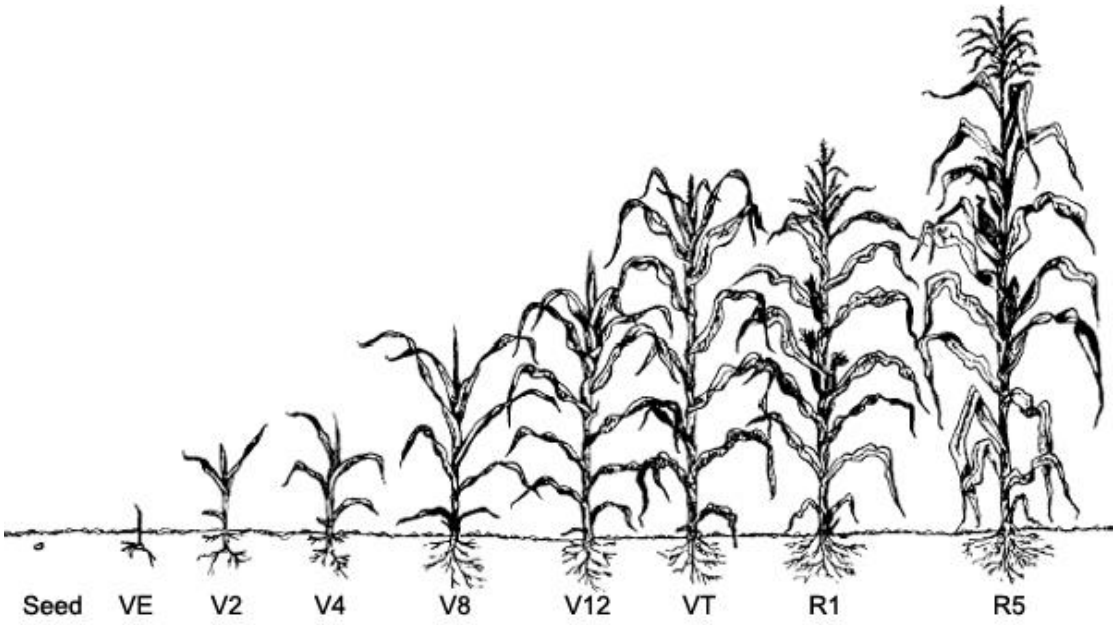

Figure 2.9 Leaves are counted from the base but exclude the growing tip. V4 growth stage selected for disease application. Diagram courtesy of Purdue University, USA. https://extension.entm.purdue.edu/fieldcropsipm/corn-stages.php\

Table 2.2 Concentration of pathogen (*Setosphaeria turcica*) spore solutions applied to plants in each experiment

Experiment	Date of application	Spore concentrations
pilot	2/08/2016	2.89×10^4
1	30/08/2016	5.06×10^4
2	5/09/2016	5.06×10^4
3	14/09/2016	1.83×10^5
4	14/09/2016	2.83×10^5
5	28/09/2016	2.83×10^5

2.4.3 Application of pathogen *S. turcica.*

The pathogen was applied when plants reached V4 stage. The spores were harvested in 0.01% Triton X100 and the concentrations used are given in Table 2.2. Five plants were treated with *S. turcica* and five treated with 0.01% Triton X100 as the control in the pilot study. Ten plants per treatment were used in the subsequent experiments with ten plants also for each cultivar and controls (Table 3.4). The second (V2) and third (V3) leaves of each plant were marked (Fig. 2.10a) and gently scratched between markings with a nail file (sandpaper). The pathogen solution was applied using sterile cotton buds soaked in the spore solution and rubbed over the entire marked area (Fig. 2.10b).

Table 2.3 Key for scoring and assessing the areas treated with *S. turcica* causing NLB disease on treated leaves.

Score	Disease symptoms
0	healthy
1	discolouration and faint lesions
2	obvious lesions
3	necrosis in area of lesion
4	necrosis at leaf tip caused by lesion
5	dead

After treatment with *S. turcica*, plants were placed in deep trays with 500 ml of water added. Separate trays were used for each isolate. The trays were then sealed with a large plastic bag (Fig. 2.11) to minimise the risk of the fungus on the seed coating being washed off into the soil. The plants were kept in the same trays for the first 48 hours at 25°C on a 16 hour light and 8 hour dark cycle to allow the pathogen to establish. The trays were incubated for 48 hours after which the plastic bags were removed. Within each tray plants were randomised, using GenStat to assign locations. The lesions were assessed by a disease score (Table 2.3). Data was recorded in excel for data analysis.

Figure 2.10 a) Black pen marks show the area that was treated with plant pathogen. Arrows indicate treated area. b) Applying the pathogen *S. turcica*.

Figure 2.11 Plants after treatment with *S. turcica* were kept humid for 48 hours to allow pathogen to germinate and grow.

2.5 Development of an insect bioassay

To test if any of the fungal isolates had an effect on *H. armigera* larvae growth or mortality when fed leaves from treated maize, a bioassay using detached leaves was developed.

Plants were grown in advance to have sufficient leaf material to feed insects. Eleven seeds were used instead of ten as germination had dropped to 90% due to the age of the seed (2014). The 21 selected isolates (Table 3.4) were applied to the seeds. The same seed coating methods as described above were used. DuPont Pioneer® cultivar P0021 seeds were used for *H. armigera* trials. P0021 was the same seed that had been consistently used in all previous assays. Plants were grown to V4 stage before leaf sections were cut off and fed to insects.

H. armigera larvae were supplied by Anne Barrington, Plant and Food Research, Mt Albert, Sandringham, Auckland. The *H. armigera* were supplied as eggs and hatching started on arrival. The larvae were placed in individual Petri dishes with supplied diet until they reached the 2nd instar stage. Each larva was weighed before being placed in a Petri dish with filter paper on the base and infused with 100 µl of sterile water to keep the leaves fresh. Three to four leaves from the treated plants, approximately 5 cm long were placed in the each Petri dish. The leaf sections were taken from the leaves closest to the stem each time to ensure the chance of endophyte presence was the strongest. Leaves were added daily and the filter paper was replaced every 2-3rd day as necessary for hygiene (Fig. 3.16). Insects were weighed weekly and deaths were recorded. The cadavers were checked for

the same fungus in the leaves used for feeding the larva and the isolate used for inoculating the plants.

Tissue samples were taken to determine if the fungal isolates could be detected as endophytes in the leaves fed to the insects. To check plants for endophytic colonisation samples were also taken from roots, stem and also the leaves for plating on PDA. The plants were harvested and processed the same as for the initial sampling methods (Chapter 2.1.1 to 2.1.2).

All statistical analyses were performed in GenStat (Version 18) with a general ANOVA test with difference (LSD) at the 5 % level.

2.6 Presence of putative endophytes in seed

It has been established that endophytes can be present in seeds (Crocker *et al*. 2016; Fisher *et al*. 1992; Logrieco and Moretti 1995). A preliminary test to establish the natural endophytes already present in the DuPont Pioneer® seeds (June 2015) was conducted to assess the presence of endophytes in the maize seeds prior to bioassays.

2.6.1 Surface sterilising of seeds

Seeds used were the two DuPont Pioneer® cultivar P0021 & 38V12, used in previous assays. Seeds were soaked for 10 mins in 0.01% triton X100-(seeds were too hard to cut otherwise) then submerged in 2% of sodium hypochlorite (bleach: Cyclone-Diversey) for five minutes, then one minute in 70% EtOH, then three washes for one minute each in dH2O. Each seed was cut in half and plated cut side down on PDA. Ten seeds were plated per Petri dish with two plates per seed line. Plates were kept in an incubator at 25°C for 5-6 days or until mycelium was seen. Subcultures established axenic cultures and these were taken through to identification as for section 2.1.4.

Chapter 3

Results

3.1 Isolation of fungi from surface sterilised maize

From the surface sterilisation of plant tissue placed onto PDA multiple genera and species of fungi grew per plate (Fig. 3.1). It was common to have up to eight morphologically different isolates on each plate, which required further subcultures to obtain pure colonies. To identify putative endophytes, isolates had to be of pure cultures culturing before plates became overgrown.

Figure 3.1 Example of different morphologies growing on PDA from maize roots.

Representatives of a total of 37 genera were identified by DNA extraction of the ITS region for both 2014 and 2015 season. In 2014, 173 isolates were recovered from 22 plants (Appendix A.1) and for a second season in 2015, a further 149 samples of fungi were isolated from twelve plants (Appendix A.2). The isolation and identification of maize from 2014 and 2015 seasons gave an unexpectedly high number of isolates belonging to four Phyla (Table 3.1). Six classes were represented in the Ascomycota phylum, two classes originated from the Basidiomycota phylum, one class from Zygomycota and one class (Oomycetes) from the Heterokonta superphylum. The majority of isolates recovered belonged to two classes of Ascomycota, Dothideomycetes (12 genera) and Sordariomycetes (16 genera). A total of 322 recovered isolates were sequenced for identification. Of these, 248 were identified to species level, 53 to genus level with 22 unable to be identified

(Appendix A.1 and A.2). There were 160 isolates positively identified as belonging to one of 73 different species (Appendix A.4).

Table 3.1 Number of recovered isolates belonging to respective Phyla

Phylum	Classes found in each phylum	Number of genera in each class
Ascomycota	Dothideomycetes	12
	Eurotiomycetes	2
	Leotiomycetes	1
	Saccharomycetes	1
	Sordariomycetes	16
	Pezizomycetes	1
Basidiomycota	Exobasidiomycetes	1
	Wallemiomycetes	1
Zygomycota	Zygomycetes	2
Superphylum-Heterokonta	Oomycetes	1

3.1.1 Microscopy results

Consensus between BLAST and UNITE was sometimes not definitive enough for the isolate to belong to one particular species. If no identifying features were found then these isolates were marked with both possibilities; e.g. isolate 32Aw-could be either *Fusarium sacchari or F. verticillioides*. Through microscopy the identifying reproductive structures or spores were examined for shape and size. For example, a collapsed sporangium (red arrow) of *Rhizopus oryzae* resembling an umbrella is seen in Figure 3.2A. The mycelium was white in colour and fast growing with long sporangiophores resulting in the grey aerial sporangium's touching the top of the Petri dish lid (Barnett and Hunter 1998; Carmichael *et al.* 1980). The sporangium structure has a columella extending from a rhizoid (green arrow) with only two hyphae showing of the rhizoid and the hyphae are aspetate (blue arrow). *Fusarium graminearum* (Fig. 3.2B) macroconidia are large spores with 5-6 septa with a tapered apical cell (Leslie *et al.* 2008). The basal end has a distinct foot. There are no microconidia produced by *F. graminearum* and this was confirmed with microscopy.

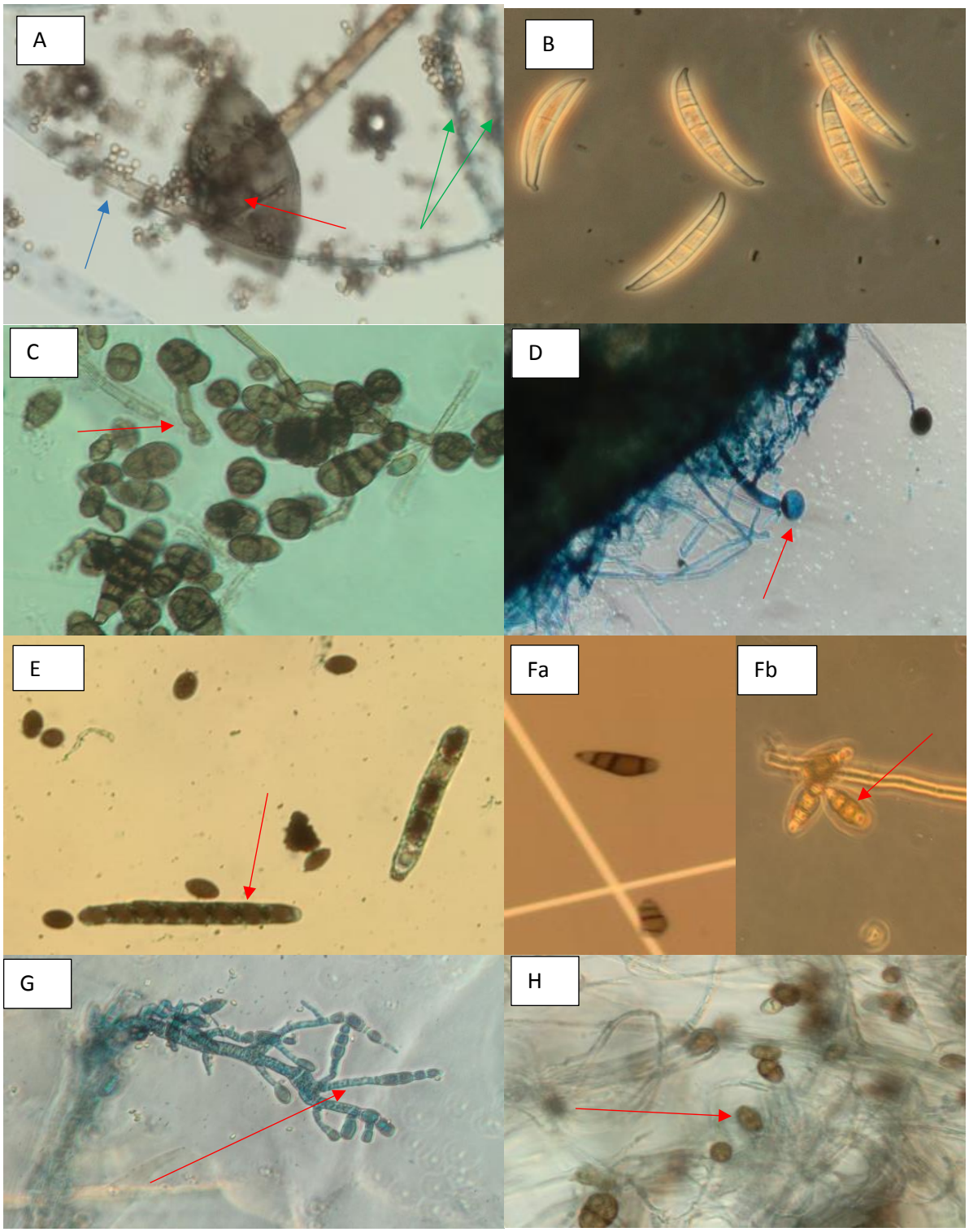

Figure 3.2 Microscopy characteristics used in identification of isolates starting from top left; A) a collapsed sporangium (indicated by red arrow) and spores of *Rhizopus oryzae* (isolate 3). 40X magnification B) Macroconidia of *Fusarium graminearum* (isolate 9A), spores. 40X magnification, C) Spores of *Alternaria alternata* (isolate 11b) with visible cell walls and conidiophore with septa indicated by red arrow. 40X magnification, D) Sporangium of *Mucor fragilis* (isolate 19) indicated by red arrow. 20X magnification, E) spores of *Sordaria fimicola* (isolate 36) red arrow indicating ascospores with eight asci present 40X magnification, Fa) the large spores of *Curvularia trifolii* (isolate 50) with Fb) the spores still attached on conidiophore (red arrow). 40X magnification, G) Chlamydospore forming in *Fusarium equiseti* (isolate 52). 20X magnification, H) spores of *Ascochyta pinodes* (isolate 68). 40X magnification.

The spores of *Alternaria alternata* (Fig. 3.2C) are large and multi cell walled with hyphae septa present (Barnett and Hunter 1998; Carmichael *et al.* 1980). *Mucor fragilis* (Fig. 3.2D) with sporangia present. The Columella is visible, the black line between the sporangiophore and the sporangium, indicated with red arrow. The spores of *Sordaria fimicola* (Fig. 3.2E) form ascospores with eight asci in each; indicated by the red arrow. Figure 3.2Fa is of *Curvularia trifolli* spores of dark colourings with three cross cell walls in only one direction and the spores have a distinct shape (centre spore). In Figure 3.2Fb the spores are still attached to the conidiophore (red arrow). A chlamydospore forming is seen in Figure 3.2G in isolate 52, *F. equiseti*. The last photo is of isolate 68, *Ascochyta pinodes* (Fig. 3.2H) spores, showing distinct septa indicated by the red arrow.

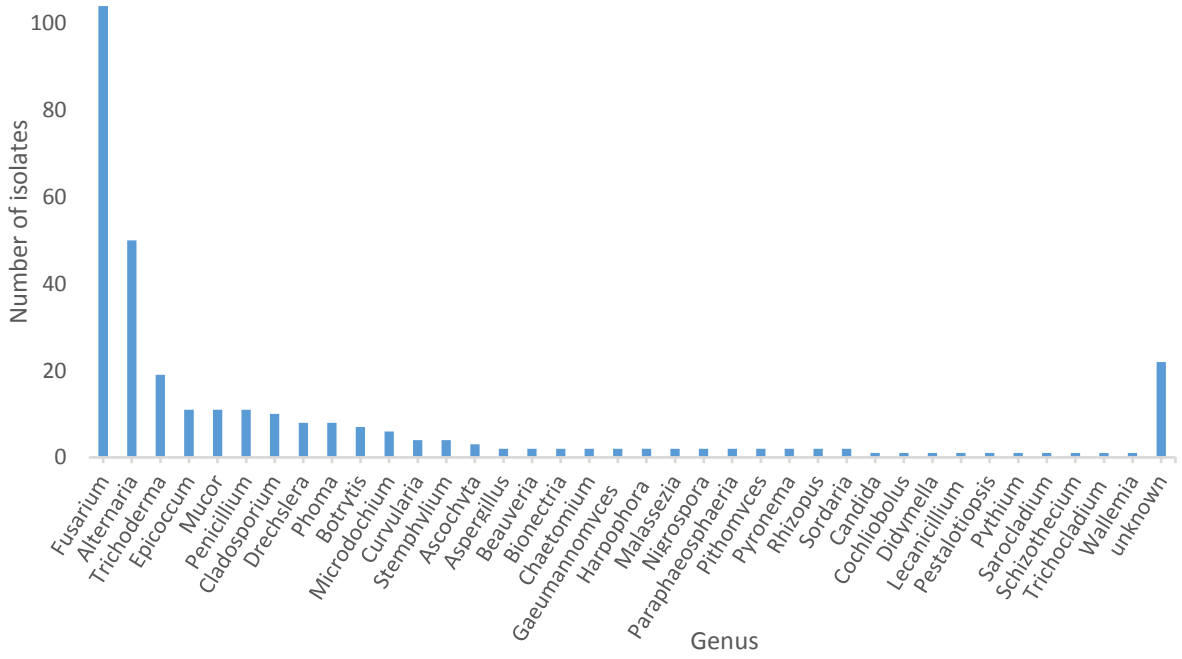

Figure 3.3 Summary of isolates cultured from each genus identified from screening of maize plants.

A total of 112 species were recovered from both growing seasons (2014 and 2015 years) from a total of 34 plants (Appendix A.3). A breakdown for both years for the number of species in each genera from the plant location and site (Appendix A.10) shows the genus *Fusarium* had the highest number of recovered isolates, with 105 isolates (Fig. 3.3) followed by *Alternaria* (48), *Trichoderma* (19) and then *Epicoccum* (13)*, Mucor* (12) and *Penicillium* isolates (11).

The results of recovered isolates were further examined to determine if differences occurred between geographical regions (Appendix A.5). Canterbury had a higher diversity with 71 species coming from 27 different genera while Waikato had 60 species coming from 20 different genera. There were 10 genera found in both Canterbury and Waikato (Table 3.2), with a further 18 found only in Canterbury, and Waikato having 10 genera not found in Canterbury.

Table 3.2 Genera found in Canterbury, Waikato or both regions.

In both regions	Canterbury only	Waikato only
Alternaria	*Aspergillus*	*Ascochyta*
Cladosporium	*Beauveria*	*Bionectria*
Drechslera	*Botrytis*	*Cochliobolus*
Epicoccum	*Candida*	*Curvularia*
Fusarium	*Chaetomium*	*Gaeumannomyces*
Mucor	*Didymella*	*Harpophora*
Penicillium	*Lewia*	*Lecanicillium*
Phoma	*Malassezia*	*Pithomyces*
Sordaria	*Microdochium*	*Nigrospora*
Trichoderma	*Paraphaeosphaeria*	*Rhizopus*
	Pestalotiopsis	
	Pyronema	
	Pythium	
	Sarocladium	
	Schizothecium	
	Stemphylium	
	Trichocladium	
	Wallemia	

The sequence and morphology (Appendix A.6) data was examined to determine if the recovered genera differed between plants. Morphology of isolates was recorded for all pure cultures but due to costs, not all were sequenced. *Fusarium* (11 species) were common in all plants from both years (Appendix A. 4). The other common genera found in both years were *Alternaria* (4 spp.)*, Epicoccum nigrum* and *Trichoderma* (5 spp.). In 2014, *Epicoccum nigrum* was found in 77% of all plants sampled, *Trichoderma* (5 spp.) was recovered in 68%, *Alternaria* (4 spp.) in 45% and *Penicillium* (3 spp.) was found in 40% of all plants sampled. In 2015, *Fusarium* (6 spp.) and *Trichoderma gamsii* was found in all 12 plants sampled (100%), *Epicoccum nigrum* was in 75%, *Alternaria* (4 spp.) was in 83%, *Penicillium* (4 spp.) was in 50% and *Cladosporium* (3 spp.) was found in 42% plants (Appendix A.4).

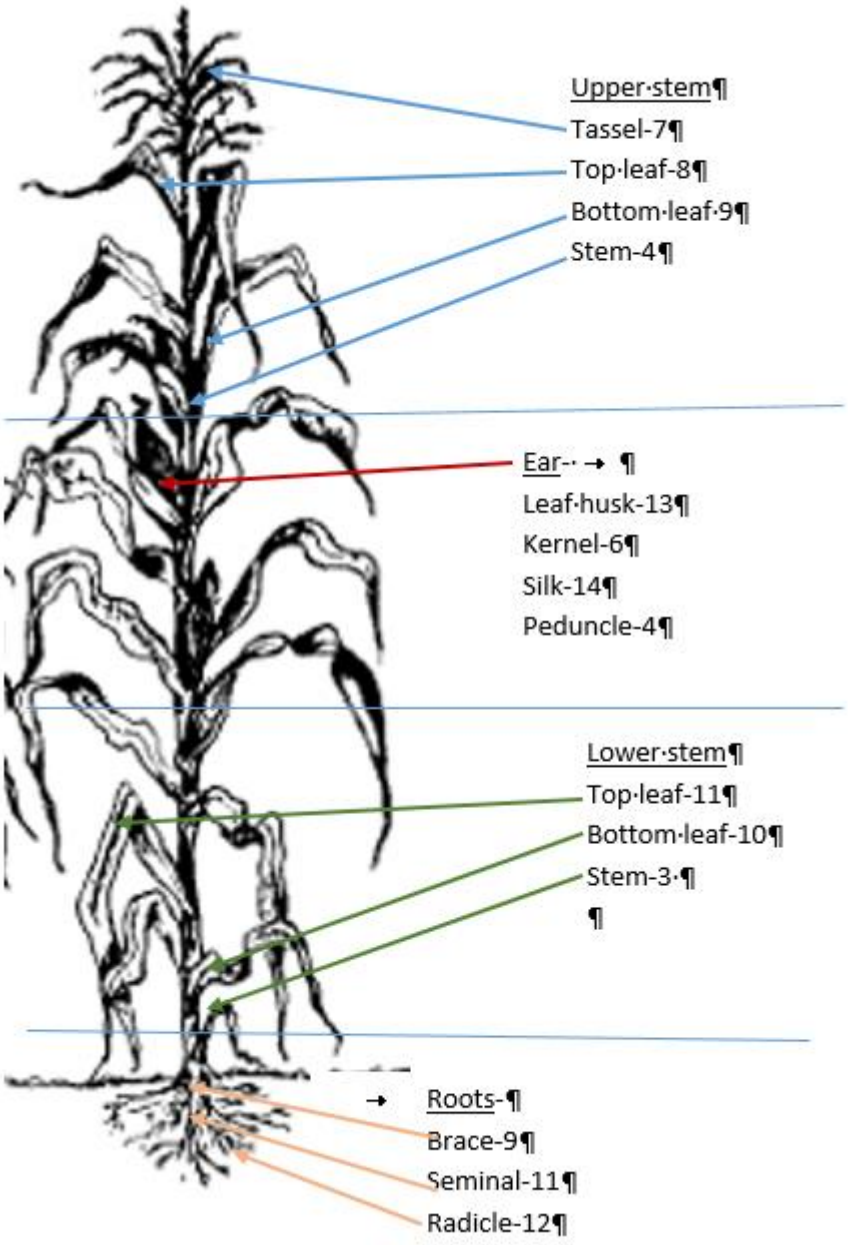

Figure 3.4 Diagram of the number of genera identified from each location as it was sampled from the maize plant. Source: https://extension.entm.purdue.edu/fieldcropsipm/corn-stages.php

Tissue samples (as sampled from the plant) were taken from maize plants from 4 locations (Fig. 3.4); roots, lower stem, upper stem and the ear, as described in Chapter 2. Within each of these locations specific sites were sampled with the number of genera found from each site (Fig. 3.4). The leaf husk from the ear location had the highest number of different genera recovered with 13 followed by the lower stem top and bottom leaves 11 and 10 genera respectively. The upper stem locations, top and bottom leaf sites, show eight and nine genera respectively.

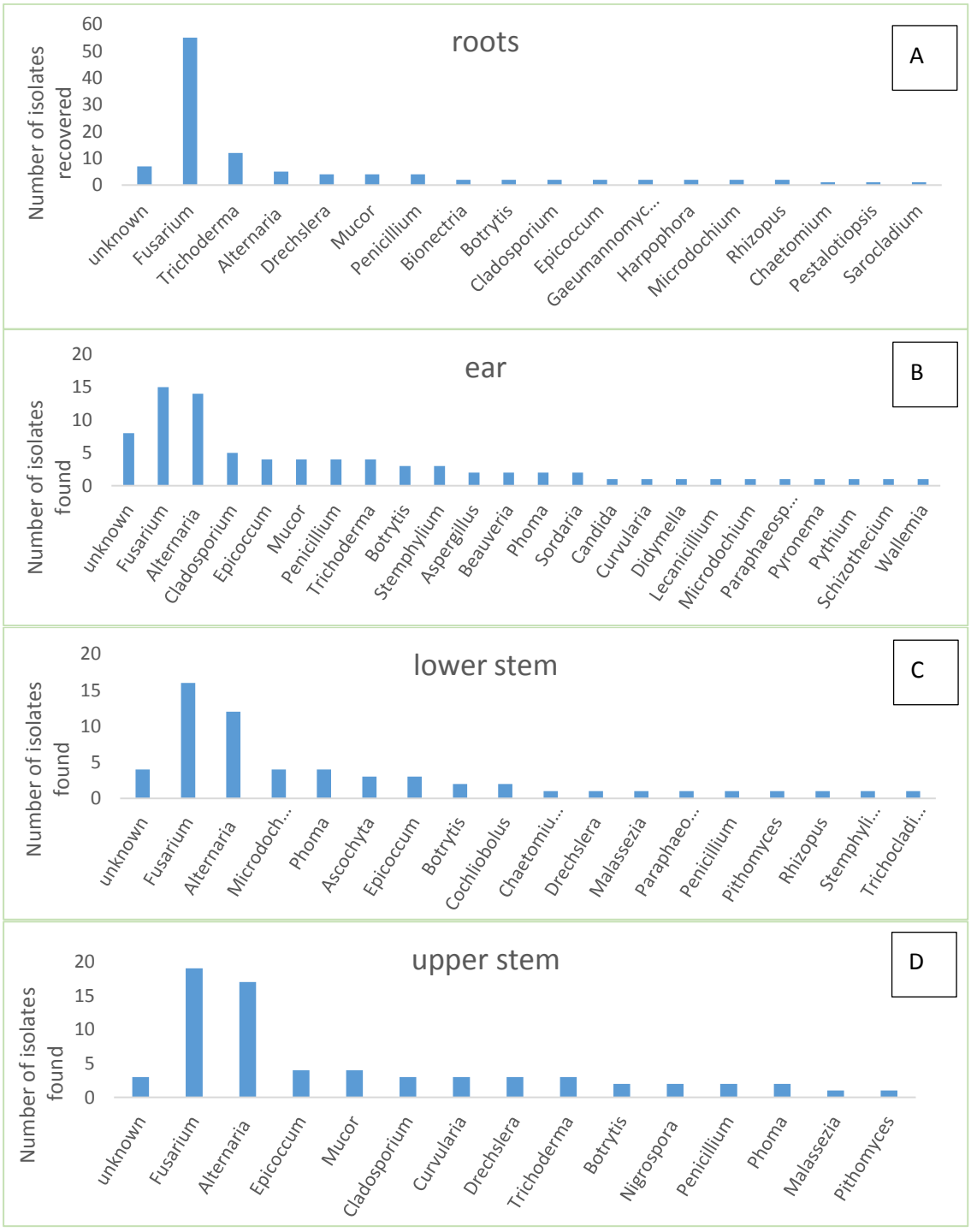

Figure 3.5 Number of isolates recovered in each genus from each location of the maize plant

The silk had 14 different genera. The number of genera was lowest from the lower stem –stem (3) and upper stem-stem (4) and the ear-peduncle had four genera. The data gave the leaf and roots sites as the highest for the number of different species recovered from the sites tested. The number of species isolated from the roots- from the brace, seminal and radicle sites show *Fusarium* was the

highest amongst the genera found (Fig. 3.5A). *Fusarium* and *Alternaria* were also the highest in the other locations sampled (Fig. 3.5B-D). The full location, plant number and site of recovered species are given in Appendix A.1 and A.2.

Data was combined to determine if endophytes were specific to specific site locations on the plant and therefore can they be considered an endophyte of that site. For example *Alternaria* was only found in leaves in a study by Fisher *et al.* (1992). Is this therefore considered a leaf endophyte? However in our study *Alternaria* spp. was found in all other sites sampled (Appendix 8). Combining the leaf data from the upper and lower stem and the leaf husk from the ear gave a different perspective of endophyte colonisation. There were 53 different species found from leaves and stems in 2014 and 2015 seasons (Appendix 9). The stems had 16 different species present combined from upper and lower stem locations.

3.1 1 Difficult isolate identifications

Some species did not present uniform colour morphology, for example-*Epicoccum nigrum* exhibited multiple morphotypes on the same media (Fig. 3.6). Figures 3.6 A & B isolates were taken from the upper stem from different plants. *Epicoccum nigrum* was found to have up to five different morphologies (Fig. 3.6 C) of yellow colourings, mustard to pinks and black for the same isolate.

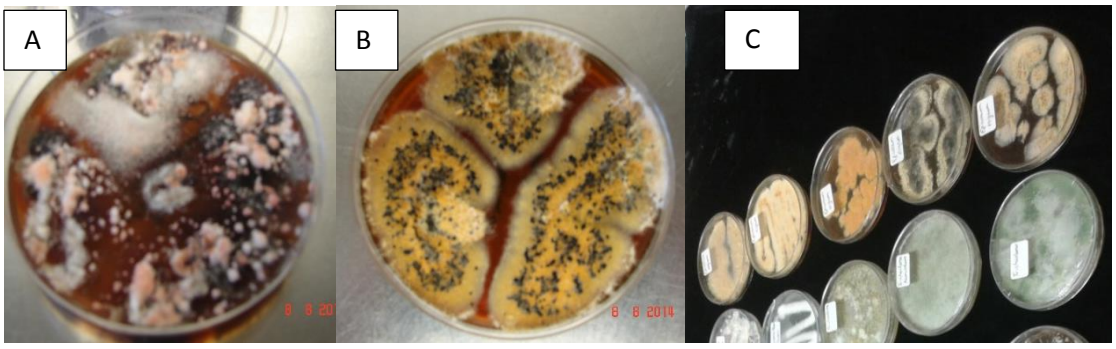

Figure 3.6 *Epicoccum nigrum* morphologies. A) from plant 4 and B) from plant 5 C) top row with 5 different morphologies of the same isolate subcultured on the same medium

Microscopy was used when sequenced results returned multiple options for species identifications, such as with isolate 71. Sequenced results from BLAST and UNITE returned identification as *Fusarium graminearum, F. culmorum* or *F. cortaderia.* From the species description given by Leslie *et al*. (2008) in The *Fusarium* Laboratory Manual, both *F. graminearum* and *F. culmorum* do not produce microconidia.

Microconidia were found (Fig. 3.7) so identification as *F. graminearum* and *F. culmorum* species could be ruled out. However there were two oval shaped spores of different lengths visible as well as one macroconidia found coloured slightly orange to brown, as seen in Figure 3.7A (circled in red), indicative of *Fusarium sterilihyphosum.* Under the microscope the macroconidia had a slight beak narrowing down to a small basal foot. A minimum of three septa could be seen. The coiled hypha (Fig. 3.7B) typical of *F. sterilihyphosum* was also found confirming the isolate to be *F. sterilihyphosum.*

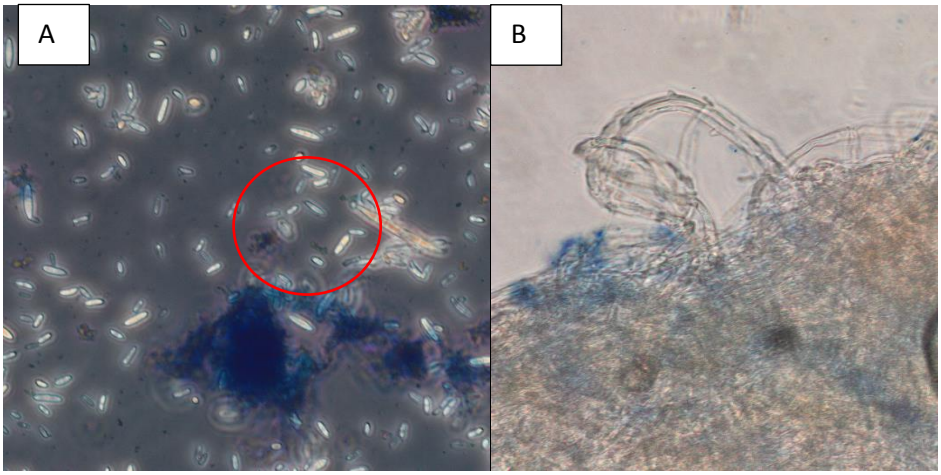

Figure 3.7 Isolate 71, microscopy confirmed identification as *Fusarium sterilihyphosum*. A) Macroconidia and microconidia spores, 40X magnification. B) Coiled hyphae, 40X magnification.

Another example using morphology for confirmation of identification was isolate 76 which was identified by ITS sequence as *F. sterilihyphosum.* Microscopy revealed the presence of napiform microconidia with a chain present in a chlamydospore. This is typical of *F. tricinctum* ruling out identification as *Fusarium sterilihyphosum* as these features are not found in this species (Fig. 3.8).

In 2015, *Trichoderma* was a common species originally recovered but when representatives were sent for sequencing there was difficulty with PCR with no bands seen, so only *T. gamsii* was identified by consensus.

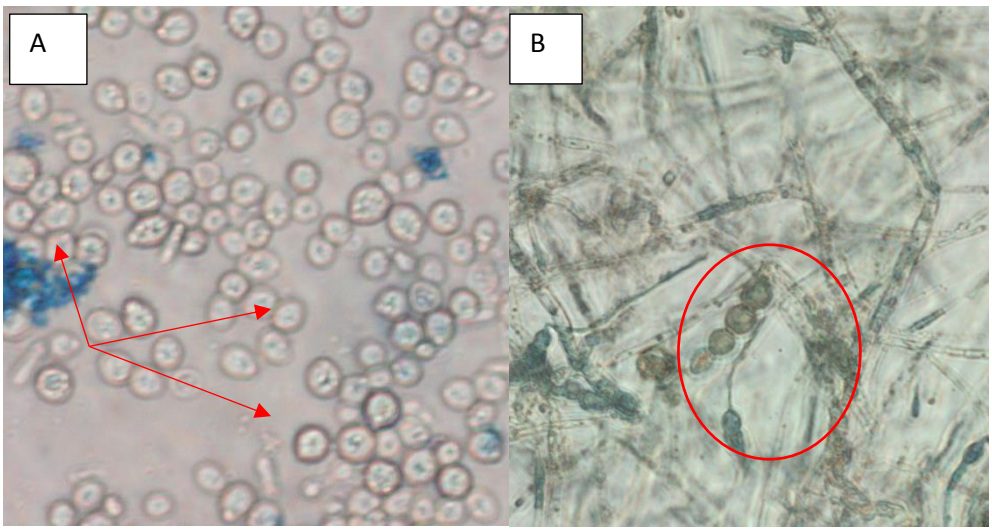

Figure 3.8 Microscopy confirmed isolate 76 was *Fusarium tricinctum* with A) distinct napiform or tear drop microconidia (red arrows) and B) chlamydospore (circled) with spores in a chain.

3.2 Dual culture results

The dual culture challenge was used to provide a rapid method to select the promising isolates for the subsequent *in planta* testing through their potential antimicrobial activity.

A total of 113 isolates were tested by the dual culture method (Fig. 3.12) (Appendix B.1). Forty-seven isolates had a positive effect by reducing the pathogen's growth (Table 3.3). Four examples of the positive effects against *S. turcica* are shown in Figure 3.9. Thirty three of these isolates reduced *S. turcica* growth compared to the unrestricted growth on control plates. Inhibition zones were seen for 10 isolates restricting the pathogen expansion. Four isolates grew over the pathogen (Table 3.3). Thirty one isolates had no effect on *S. turcica* or the pathogen grew more than the control plates (Fig. 3.10). Isolates which did not impact on the growth of *S. turcica* were not taken any further, except for *B. bassiana* (Fig. 3.10B). Of the 47 isolates, with most impact on *S turcica,* 21 were used for further testing for disease and insects assays (Table 3.4).

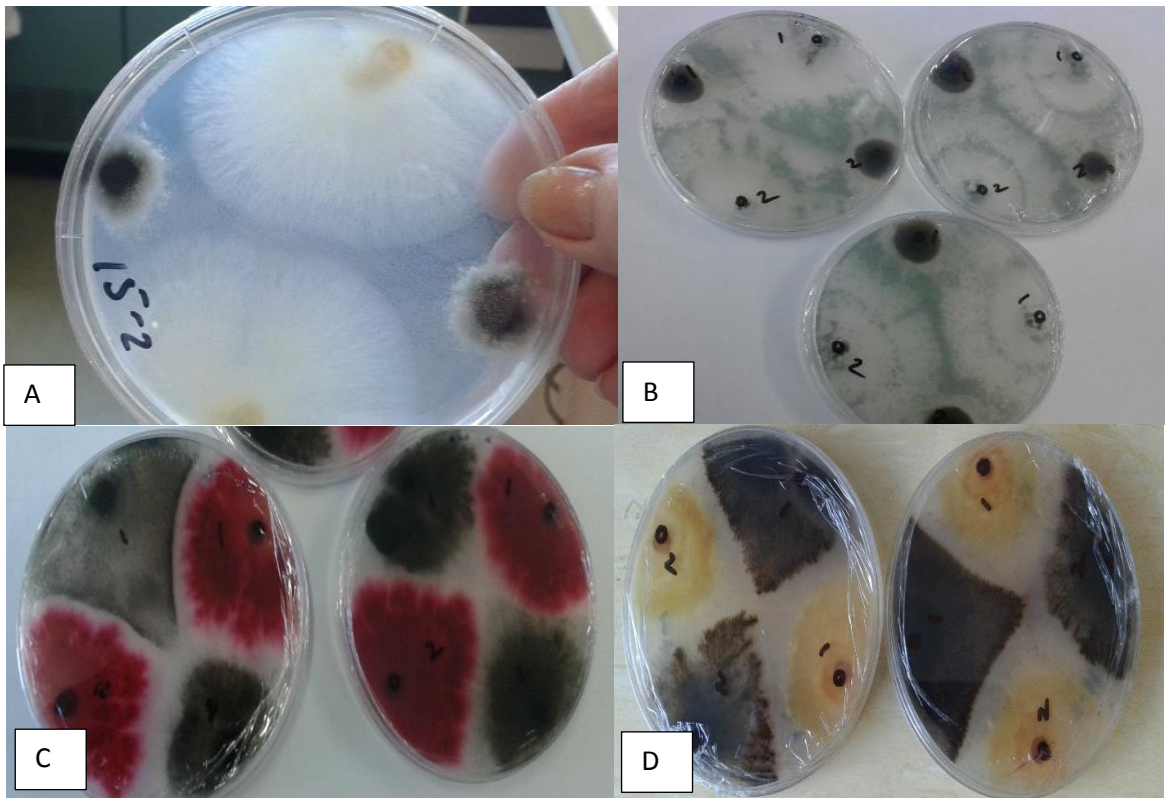

Figure 3.9 Dual culture of putative endophytes showing impact the plant disease *S. turcica* A) *Mucor hiemalis* (15) restricting the growth of the *S. turcic*a, B) *Trichoderma atroviride* (95-8) showing inhibition zones around *S. turcica* and where the isolate plugs meet. C) *Fusarium graminearum* (9A) showing definitive inhibition zones-and D) *Fusarium avenaceum (*60A) also showing distinct inhibition zones.

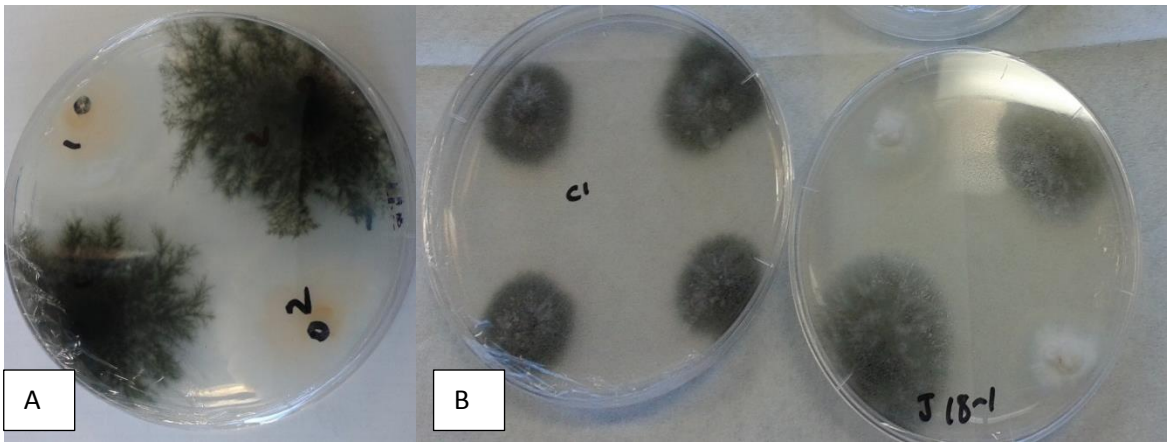

Figure 3.10 *Bionectria ochroleuca* (isolate 13) and B) *B. bassiana* (isolate J18) increased the pathogen growth (right) compared to control (left-c1).

Table 3.3 Summary of isolates which had an effect on the plant pathogen, *Setosphaeria turcica* in dual culture assays using putative endophytes.

Isolate number	Isolate reduced pathogen growth	Isolate number	Isolate reduced pathogen growth
3	*Rhizopus oryzae*	99	*Epicoccum nigrum*
8	*Fusarium culmorum*	102	*Phoma herbarum*
10	*Mucor hiemalis*	107	*Phoma herbarum*
12	*Alternaria tenuissima*	108	*Penicillium purpuroquenum*
35	*Fusarium proliferatum*	118	*Mucor fragilis*
36	*Sordaria fimicola*	125	*Aspergillus ochraceus*
37	*Fusarium sterilihyphosum*	127	*Didymella* sp.
42	*Botrytis cinerea*	129	*Fusarium equiseti*
46	*Trichoderma koningiopsis/gamsii*	130	*Botrytis cinerea*
55	*Trichoderma hamatum*	134	*Penicillium adametzioides/spinulosum*
82	*Fusarium avenaceum*	140	*Lewia infectoria/ Alternaria rosae*
85A	*Trichoderma koningiopsis/atroviride*	143	*Trichoderma koningiopsis/atroviride*
86	*Lecanicillium lecanii*	144	*Fusarium oxysporum*
95-2	*Trichoderma koningiopsis/gamsii*	152	*Stemphylium globuliferum*
95-5	*Trichoderma hamatum*		
95-6	*Trichoderma hamatum*		
95-9	*Trichoderma koningiopsis*		

Isolate number	Isolate caused an area of zero growth or an inhibition zone	Isolate number	Isolate overgrew pathogen
9A	*Fusarium graminearum*	95-3	*Trichoderma* sp.
51	*Fusarium proliferatum*	95-7	*Trichoderma harzianum*
60A	*Fusarium avenaceum*	137	*Wallemia sebi*
60B	*Fusarium avenaceum*	143	*Trichoderma koningiopsis/atroviride*
95-8	*Trichoderma atroviride*		
95-10	*Trichoderma asperellum*		
101B	*Fusarium sambucinum/venenatum*		
101	*Fusarium sambucinum/venenatum*		
114	*Fusarium oxysporum*		
119	*Penicillium brasilianum*		
139	*Fusarium oxysporum*		

Table 3.4 Isolates selected for testing in *planta* for disease assays and for insect bioassays by seed coating.

Isolate number	*Setosphaeria turcica* pathogen or triton applied to leaves
3	*Rhizopus oryzae*
15	*Mucor hiemalis*
19	*Mucor fragilis*
J21	*Beauveria bassiana*
24	*Epicoccum nigrum*
36	*Sordaria fimicola*
43A	*Mucor racemosus*
50A	*Curvularia trifolii*
51	*Fusarium proliferatum*
53a	*Fusarium acuminatum*
60A	*Fusarium avenaceum*
80	*Penicillium olsonii*
86	*Lecanicillium lecanii*
95-8	*Trichoderma atroviride*
107	*Phoma herbarum*
108	*Penicillium purpurogenum*
119	*Penicillium brasilianum*
125	*Aspergillus ochraceus*
129	*Fusarium equiseti*
134	*Penicillium adametzioides/spinulosum*
137	*Wallemia sebi*

3.3 *In planta*

To recap the methods for *in planta* testing, maize seed were coated with selected isolates and then grown for two months. The pathogen *S. turcica* was applied to pre-treated maize leaves and scored on the area of necrosis. Twenty-one putative endophyte isolates were tested (Table 3.4). Each isolate-treated plant was compared to the disease incidence on control plants with no endophytic fungi added (Fig. 3.12). Above the green line the plant had an increase in disease level compared to controls and below the line; the isolate treatment resulted in reduced disease. Addition of fungi to the seed in cultivar P0021 resulted in 9 out of 21 isolates with reduced disease and for cultivar 38V12, 13 out of 21 plants were below the ratio of 1. The ratio of 1 was the control average level of disease score when the seed was treated with just Triton X100.

Disease symptoms started showing after seven days of growth from inoculation. The disease score was visible at approximately 7 - 10 days and the measurements taken 10 - 12 days after application (Fig. 3.14). Disease showed as patches of necrosis tissue as yellowing in colour to completely dead tissue (Fig.

3.15). The ratio for disease severity for fungal inoculated plants were compared to the control plants with each plant being measured by a score of 1-5 scale (Table 2.4) for level of disease severity for both cultivars P0021 and 38V12 and an average given for each treatment (Appendix C1).

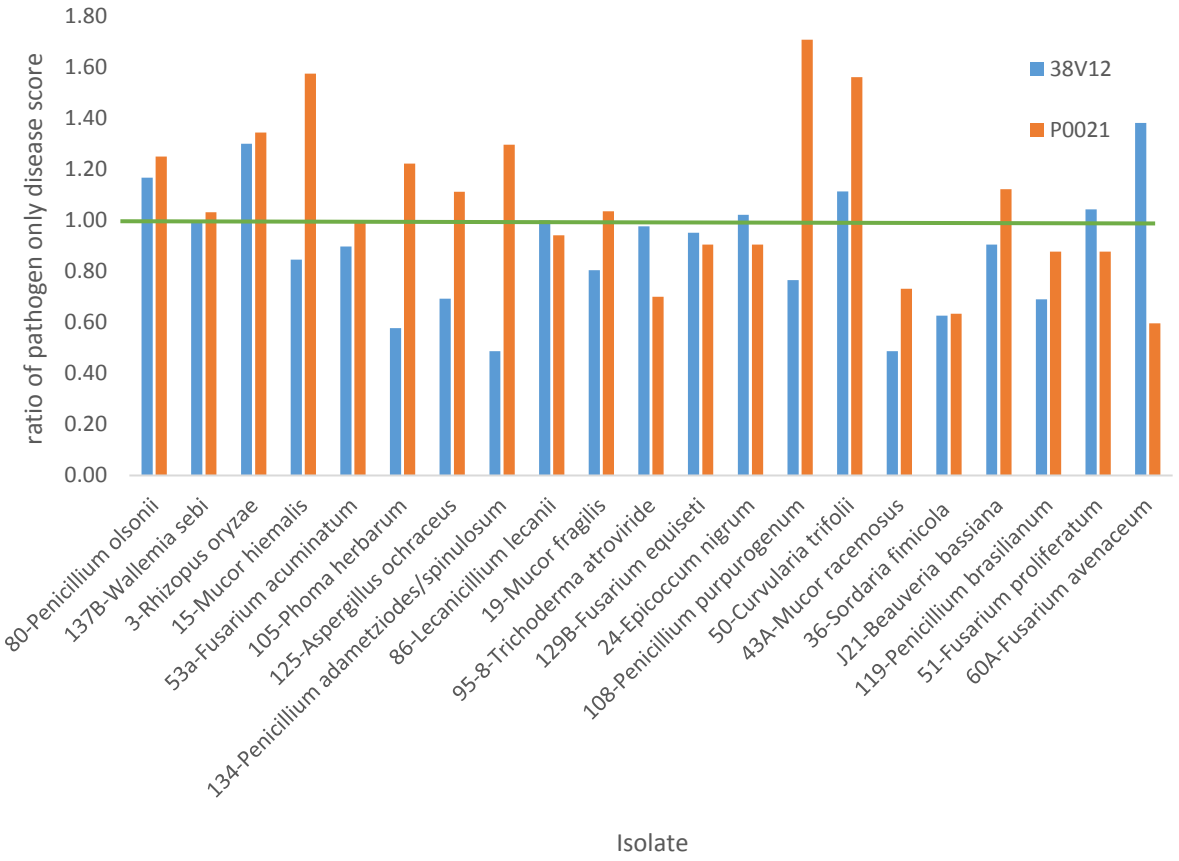

Figure 3.11 Effect of *S. turcica* on maize leaves pre-treated with selected fungal isolates or untreated (control). Two cultivars P0021 and 38V12 were scored with a disease severity rating (0-5) and the ratio of disease severity determined compared to control plants.

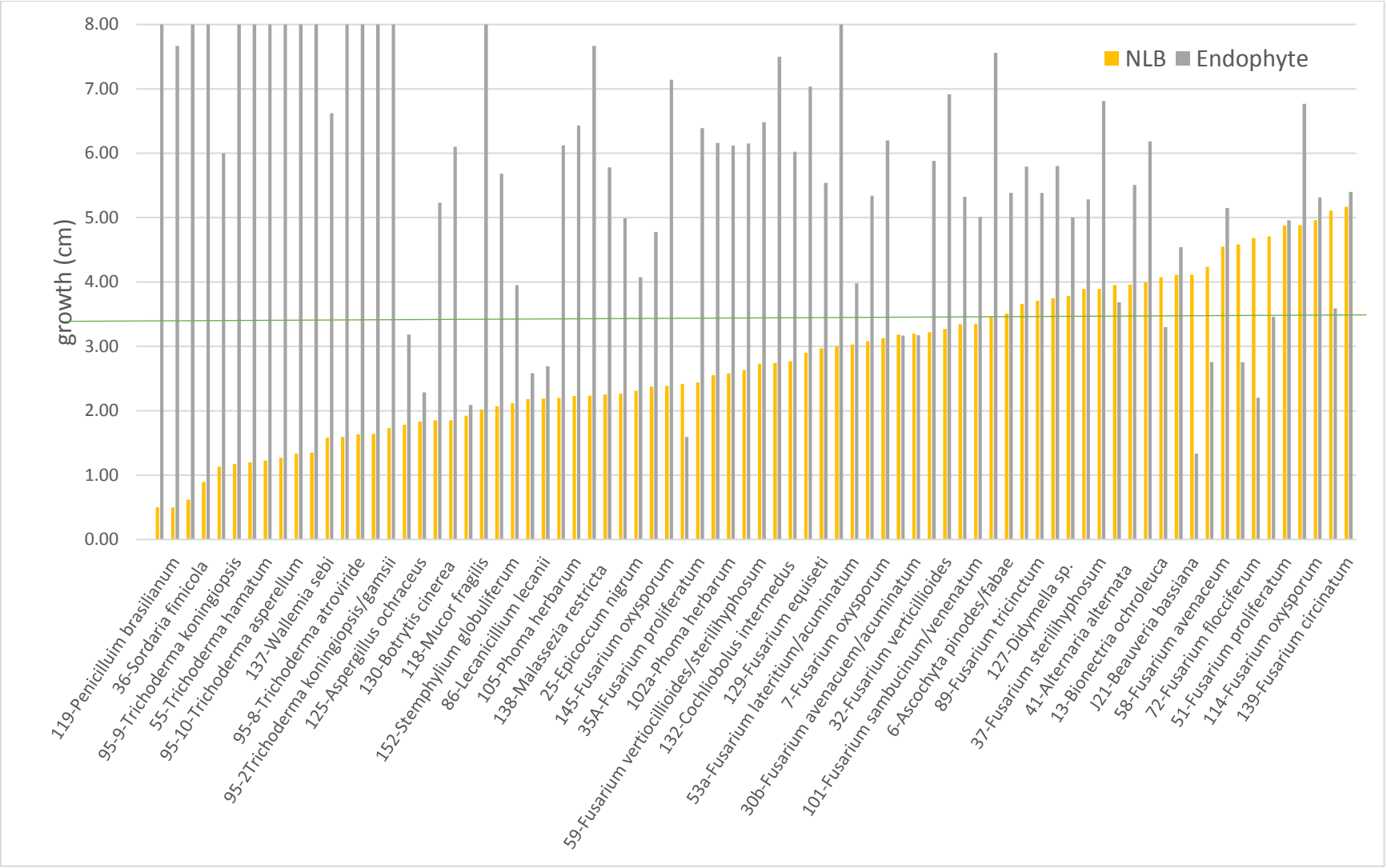

Figure 3.12 Growth of *Setosphaeria turcica* and individual putative endophyte isolates in dual culture tests. Control = *S. turcica* alone. Green line represents the average control (*S. turcica* growth alone) measurement of all assays at 7 days. Maximum possible growth is 8 cm- the width of a Petri dish (Refer to Appendix B.1 for all 79 isolate names).

The control level of disease is shown by the green line in Figure 3.12 at 1. All the isolate scores were averaged to determine effect of cultivar against disease. Cultivar 38V12 averaged a disease score of 0.89 showing less disease than the control. P0021 had a disease score of 1.1 indicating the latter cultivar displayed slightly increased disease levels compared to the control (Appendix C.1). Five isolates scored below 1, for both cultivars: *Sordaria fimicola* (isolate number 36)*, Mucor racemosus* (43A)*, Trichoderma atroviride* (95-8), *Penicillium brasilianum* (119) and *Fusarium equiseti* (129B). The next three isolates to have a positive effect for disease incidence reduction were *Fusarium acuminatum* (53A), *Mucor fragilis* (19)*,* and *Fusarium proliferatum* (51), making eight isolates for potential testing in the future.

A comparison of *S. turcica* applied to leaves from pre-treated maize seeds by selected isolates or control (untreated) (Fig. 3.13) using analysis of variance (ANOVA) for assays A-E was performed. Isolates that gave a significant reduction in disease score were; for cultivar P0021-*Trichoderma atroviride* (95-8), *Aspergillus ochraceus* (125) *Phoma herbarum* (105), *Fusarium acuminatum* (53A*)* and *Fusarium avenaceum* (60A). In cultivar 38V12, isolates that reduced the disease score were; *Mucor racemosus* (43A), *Phoma herbarum* (105), *Aspergillus ochraceus* (125), *Penicillium adametzioides/spinulosum* (134) and *Mucor fragilis* (19). Two isolates resulted in a significant increase in disease score; *Rhizopus oryzae* (3) in cultivar 38V12 and *Phoma herbarum* (108) in cultivar P0021.

The NLB disease is visible in Figure 3.14. The pathogen effect on the V2 leaf (Fig. 3.14A-left leaf) compared to the leaf at V3 (Fig. 3.14A-right leaf) show a difference in disease score; V2=5, complete necrosis and death, and V3=3-showing necrotic areas (Disease score Table 2.4). A comparison of a pathogen treated leaf compared to the healthy control, 0.01 % Triton treated, is seen in Figure 3.14B. The growing plant with V2 and V3 treated leaves with visible disease on leaves is evident in Figure 3.15. The seed was treated with isolate 129 B-*Fusarium equiseti*.

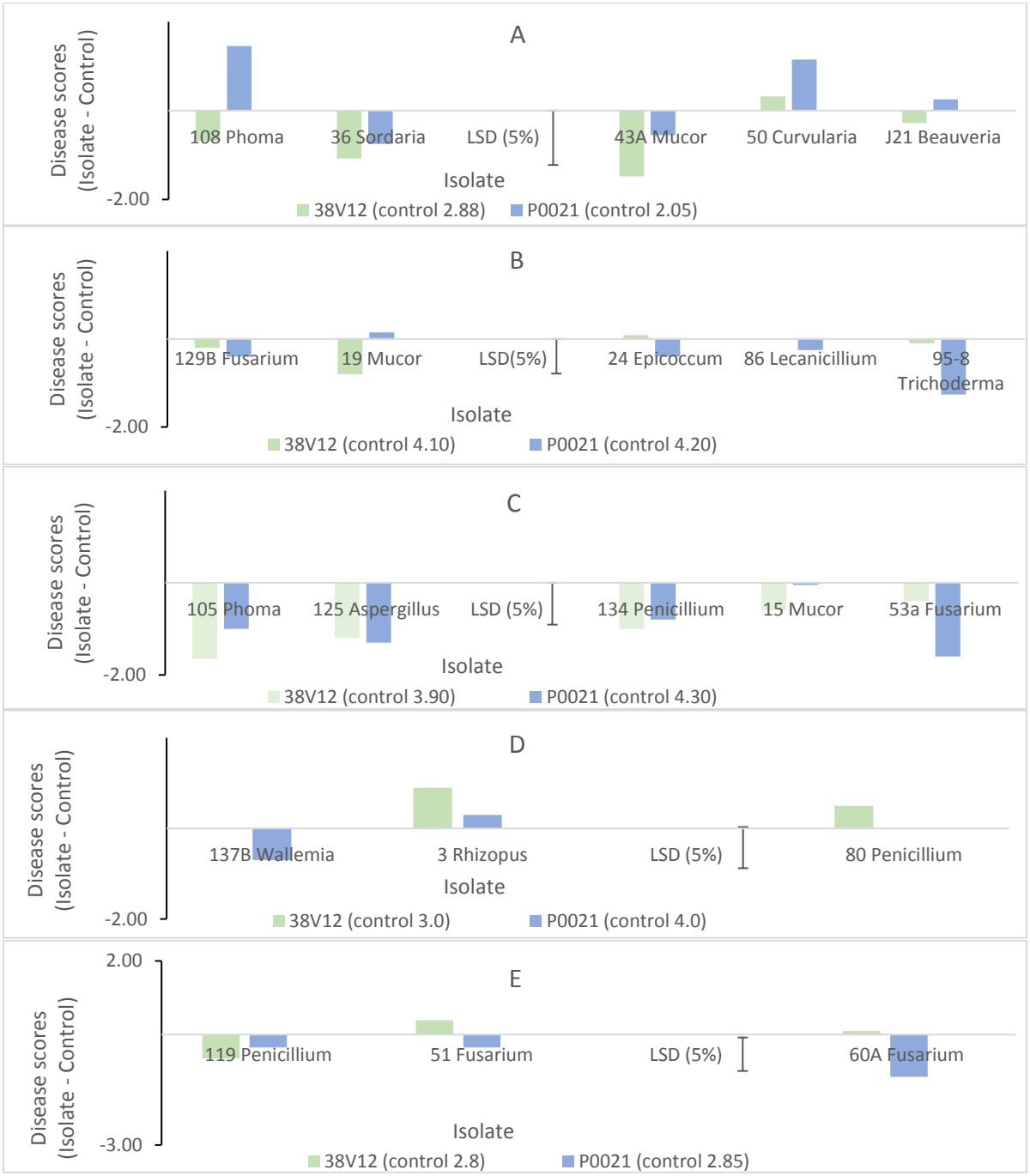

Figure 3.13 *In planta* effects on plant disease severity in assays A- E with different isolates tested for each assay. In each graph the difference in disease score between each isolate and the control is presented for each cultivar. The mean score for the controls are given in the key for each cultivar and assay. The 5% least significant difference (LSD) is given for each assay; mean differences which are greater in numerical value than the LSD (5%) represent isolates that differ significantly ($p<0.05$) from the control, using the unrestricted LSD procedure (Saville 1990). Isolates tested were from top; Figure 3.13A- *Phoma herbarum* (108), *Sordaria fimicola* (36), *and Mucor racemosus* (43A), *Beauveria bassiana* (J21). Figure 3.13B- *Fusarium equiseti* (129B), *Mucor fragilis* (19), *Epicoccum nigrum* (24), *Lecanicillium lecanii* (86) and *Trichoderma atroviride* (95-8) and Figure 3.13C-*Phoma herbarum* (105), *Aspergillus ochraceus* (125), *Penicillium adametzioides/spinulosum* (134), *Mucor hiemalis* (15) and *Fusarium acuminatum* (53A). Figure 3.13D- *Wallemia sebi* (137B), *Rhizopus oryzae* (3), *Penicillium olsonii* (80) and Figure 3.13E- *Penicillium brasilianum* (119), *Fusarium proliferatum* (51) and *Fusarium avenaceum* (60A).

Figure 3.14 A) Cultivar 38V12 showing pathogen treated leaves from plants grown from seed coated with *Epicoccum nigrum* (24). Score rating for V2 (small leaf) was five. The V3 (large leaf) (right) scored three. The second photo B) shows the left leaf from pot 495. The cultivar used was 38V12, and the seed treated with-*Fusarium proliferatum* (isolate 51). The treatment applied to the leaf was 0.01 % Triton X 100 (control). The right leaf was from pot 457, cultivar P0021, with the pathogen applied to seed treated of-*Penicillium brasilianum* (119). The score rating for the right leaf was three.

Figure 3.15 Maize leaves V2 and V3, from cultivar 38V12, showing visible signs of disease extending beyond marked lines of pathogen application. Seed was treated using *Fusarium equiseti* (129B). V2 score =5 (necrosis of whole leaf) and V3 score =4 (leaf tip not showing necrosis but visible lesion within the marked application area).

3.4 Insect bioassays

Weights of treated *H. armigera* larvae were measured weekly over three weeks (Table 3.5). The larvae were fed treated leaves (Fig. 3.16) as described in Chapter 2.5. The average weight gain for each surviving larvae was statistically compared to the control and other isolates tested. Statistical analysis indicated that the average weight gain of surviving larvae on several isolate-treated plants was significantly different compared to larvae fed untreated control plants (Table 3.5). After week one, larvae that were fed on plants from 11 isolate treatments showed significantly less weight gain compared to controls. In week two only one isolate treatment resulted in differential feeding and in week three, five isolate treatments were significantly different to the control, although only two isolates significantly reduced live weight gain. In the second week a comparison was done between the average weight at week two compared to the initial weight (week zero) (Table 3.5). Larvae feeding from seven isolates showed a significant difference from control. These isolates were *Fusarium avenaceum* (60A*), Trichoderma atroviride* (95-8), *Phoma herbarum* (105), *Penicillium brasilianum* (119), *Aspergillus ochraceus* (125), *Fusarium equiseti* (129B) and *Penicillium adametzioides/spinulosum* (134). In week three the comparison to week zero showed two isolates had a significant effect, *Penicillium brasilianum* (119) and *Fusarium equiseti* (129B).

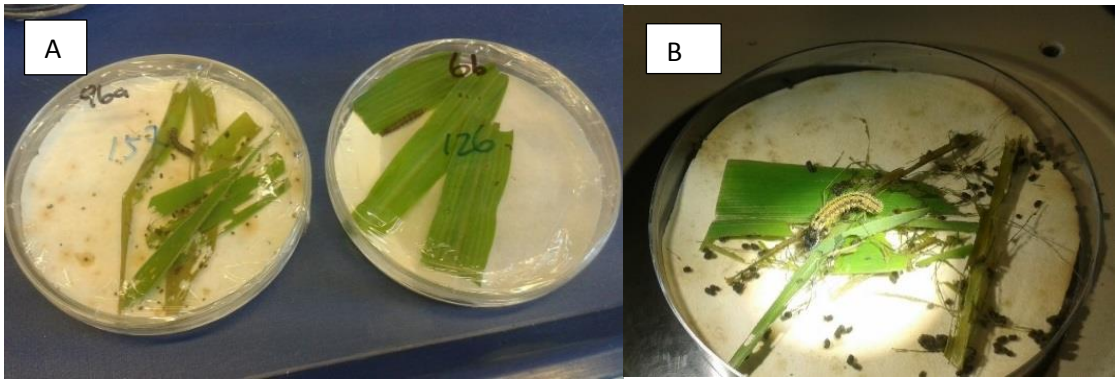

Figure 3.16 *Helicoverpa armigera* feeding in detached leaf assay. A) Left plate where showing when more food was added and the right plate had been freshly set up. B) *H. armigera* caterpillar after 14 days feeding.

Mortality was recorded for each isolate weekly and Abbott's formula was used to correct for untreated control mortality (Table 3.6). In Figure 3.18, the isolates were grouped into levels of mortality from the highest mortality (80% uncorrected mortality) in the first group on the left to the least number of deaths (20%) on the right. Seventeen of the isolate treatments had higher mortality compared to the control; however only seven isolates had over 50% mortality (Table 3.6). Mortality of insects after being fed on leaves from endophyte-treated plants showed seven isolates significantly different from the untreated control; *Rhizopus oryzae* (3)*, Fusarium acuminatum* (53A)*, Penicillium adametzioides/spinulosum* (134)*, Epicoccum nigrum* (24)*, Sordaria fimicola* (36)*, Mucor racemosus* (43A) and *Fusarium proliferatum* (51) (Chi squared ($\chi 2$) at 5% critical value=3.84).

Table 3.5 *Helicoverpa armigera* live weight gains (LWG) over three weeks fed maize leaves with seeds coated with respective isolates.

Treatment/Isolate number and name	Number of larvae alive week 1	LWG (mg) week 1- week 0 (initial weight)	Number of larvae alive week 2	LWG (mg) wk 2 - wk 1 (Average weight gain)	LWG (mg) wk 2 - wk 0 (Average weight gain)	Number of larvae alive week 3	LWG (mg) wk 3 - wk 2 (Average weight gain)	LWG (mg) wk 3 - wk 0 (Average weight gain)
Control (C)	20	441	19	738	1177	15	695	1906
3-Rhizopus oryzae	10	438	5	936#	1410#	4	108*	1720
15- Mucor hiemalis	10	456	9	833	1284	5	518	1945
19- Mucor fragilis	10	460	8	842	1313	7	600	2010
J21- Beauveria bassiana	10	412	10	814	1226	7	422	1704
24- Epicoccum nigrum	10	526	7	607	1154	2	635	1740
36-Sodoria fimicola	9	420	7	815	1270	2	423	2003
43 -Mucor racemosus	9	476	9	762	1238	3	844	2293#
50A-Curvularia trifolii	10	368	10	727	1094	5	369#	1637
51-Fusarium proliferatum	10	532*	7	758	1344	3	444	1730
53A-Fusarium acuminatum	10	397	8	808	1204	2	462	1815
60A-Fusarium avenaceum	10	324*	10	622	946*	8	1013*	1959
80-Penicillium olsonii	10	329*	10	668	996	6	930	1957
86-Lecanicillium lecanii	10	271*	9	668	958	8	923	1859
95-8 Trichoderma atroviride	10	362*	10	534*	896*	8	738	1631
105-Phoma herbarum	10	323*	10	603	926*	7	851	1747
108-Penicillium purpurogenum	10	288*	10	671	959	7	246*	1195*
119-Penicillium brasilianum	11	331*	11	612	943*	7	589	1509*
125-Aspergillus ochraceus	10	283*	10	607	890*	6	787	1696
129B-Fusarium equiseti	10	316*	10	602	918*	6	674	1544
134-Penicillium adametzioides/spinulosum	9	265*	9	558	824*	3	1180*	2027
137B-Wallemia sebi	10	324*	10	647	971	8	1023*	1979

LSD (5%) values for different comparisons and different numbers of live larvae (n1 versus n2 live larvae)

Comparison	n1 v n2	LSD	n1 v n2	LSD	LSD	n1 v n2	LSD	LSD
control vs isolate (max reps)	20 v 10	87	19 v 10	197	221	15 v 8	287	349
control vs isolate (min reps)	20 v 9	90	19 v 5	254	284	15 v 2	493	600
isolate (max reps) vs isolate (max reps)	10 v 10	101	10 v 10	226	253	8 v 8	327	398
isolate (max reps) vs isolate (min reps)	10 v 9	103	10 v 5	277	310	8 v 2	517	630
isolate (min reps) vs isolate (min reps)	9 v 9	106	5 v 5	319	358	2 v 2	654	797

not significant as only 5 or 3 larvae alive

*Isolates that differ significantly ($p<0.05$) from control, using the unrestricted LSD procedure (Saville 1990).

Table 3.6 Mortality of *Helicoverpa armigera*, fed on maize leaves from seed coated plants with respective isolates from 21 fungi.

	Isolate used in treatment	Number larvae dead	Number larvae alive	Mortality (%)	% dead (Abbotts corrected)	Chi squared (χ2) (Isolate vs Control)	Statistical significance
	Control (C)	5	15	25	-	-	-
1	3 *Rhizopus oryzae*	8	2	80	73.33	6.12	<5%*
2	53A-*Fusarium acuminatum*	8	2	80	73.33	6.12	<5%*
3	134-*Penicillium adametzioides/spinulosum*	7	3	70	60	3.9	<5%*
4	24- *Epicoccum nigrum*	7	3	70	60	3.9	<5%*
5	36-*Sodoria fimicola*	7	3	70	60	3.9	<5%*
6	43A *Mucor racemosus*	7	3	70	60	3.9	<5%*
7	51-*Fusarium proliferatum*	7	3	70	60	3.9	<5%*
8	15-*Mucor hiemalis*	5	5	50	33.33	0.19	n/s
9	50A-*Curvularia trifolii*	5	5	50	33.33	0.19	n/s
10	80-*Penicillium olsonii*	4	6	40	20		n/s
11	105-*Phoma herbarum*	4	6	40	20		n/s
12	108-*Penicillium purpurogenum*	4	6	40	20		n/s
13	125-*Aspergillus ochraceus*	4	6	40	20		n/s
14	19-*Mucor fragilis*	3	7	30	6.67		n/s
15	J21-*Beauveria bassiana*	3	7	30	6.67		n/s
16	119-*Penicillium brasilianum*	3	7	30	6.67		n/s
17	129B-*Fusarium equiseti*	3	7	30	6.67		n/s
18	60A-*Fusarium avenaceum*	2	8	20	-6.67		n/s
19	86-*Lecanicillium lecanii*	2	8	20	-6.67		n/s
20	95-8 *Trichoderma atroviride*	2	8	20	-6.67		n/s
21	137B-*Wallemia sebi*	2	8	20	-6.67		n/s

Key

χ2 (5% critical value)=3.84 *significantly different from control

χ2 (1% critical value)=6.63 n/s=not significant

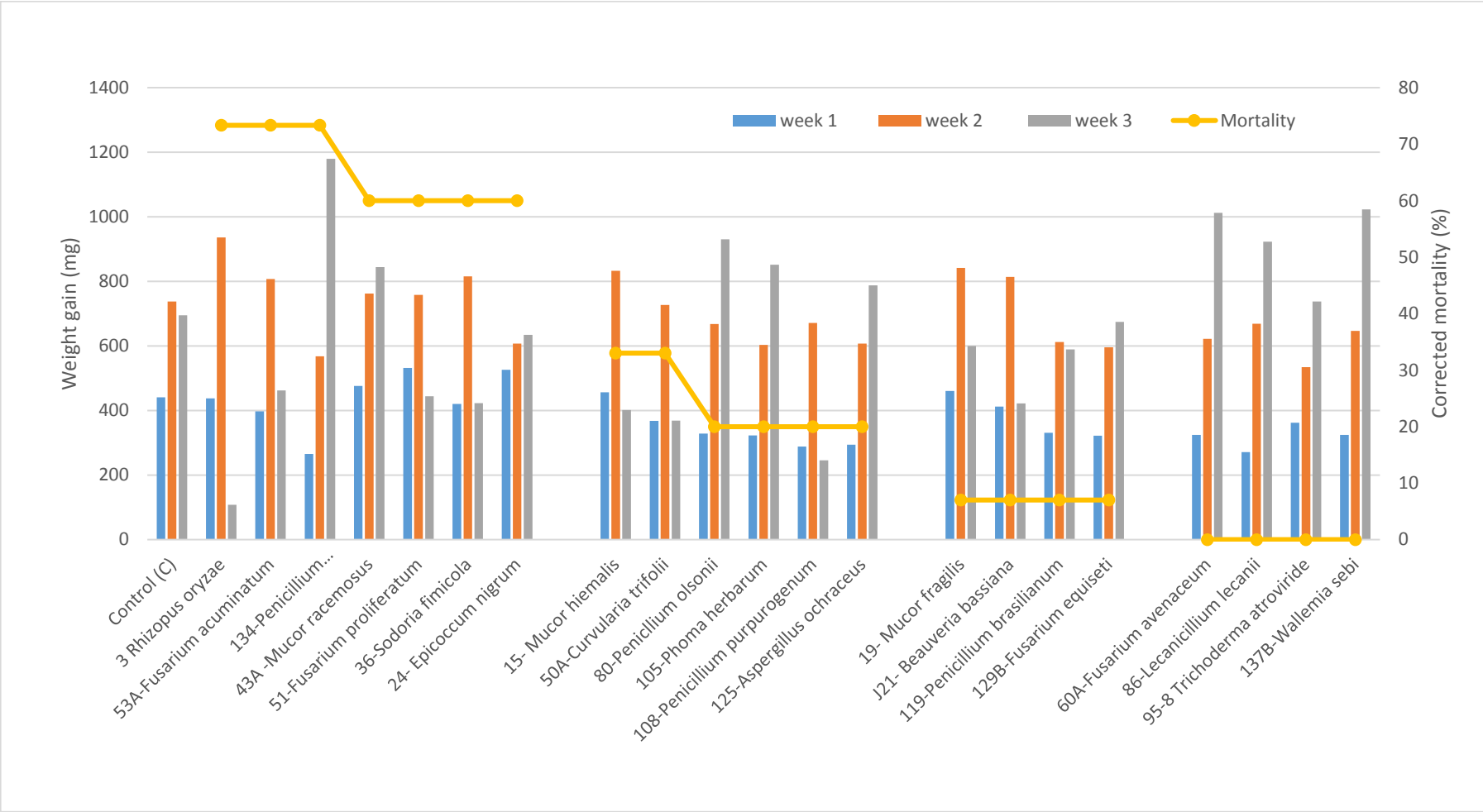

Figure 3.17 Isolates grouped by Abbott's corrected mortality of *Helicoverpa armigera* larvae after being fed on leaves of maize grown from treated seeds. Weights were recorded weekly for three weeks.

3.5 Endophyte recovery from plants

Maize tissues from plants used in *H. armigera* leaf assays were surface sterilised to determine if applied fungi could be recovered on culture plates (the same method as in the sampling described in Chapter 2.1). A total of 295 plates, containing root, stem and leaf from plants were plated from seed treated plants used in Helicoverpa assays. Twelve isolates (Table 3.7) identified by morphology, were confirmed in plants treated with the inoculated fungus while eight isolates were not found in any samples. The isolates not found were; *Mucor fragilis* (19), *Epicoccum nigrum* (24), *Fusarium proliferatum* (51) *Penicillium purpurogenum* (108)*, Penicillium brasilianum* (119)*, Aspergillus ochraceus* (125), *Fusarium equiseti* (129B) and *Wallemia sebi* (137B)*.* Interestingly, *Epicoccum nigrum* (isolate 24) treatment resulted in high mortality of caterpillars whereas the other five isolates not recovered were in the lowest mortality group. Only *R. oryzae* was recovered from a dead *H. armigera.*

Table 3.7 Recovery of inoculated isolates from maize plants used for feeding *Helicoverpa armigera* and the number of dead *H. armigera*.

	Dead *Helicoverpa*	Roots	Stem	Leaf	Total samples with endophytes
3-Rhizopus oryzae	1			1	2
15- Mucor hiemalis				1	1
36-Sordaria fimicola		1		4	5
43A -Mucor racemosus				4	4
53A-Fusarium acuminatum		2	1	2	5
60A-Fusarium avenaceum			2		2
80-Penicillium olsonii		2		1	3
86-Lecanicillium lecanii		1		2	3
95-8 Trichoderma atroviride				1	1
105-Phoma herbarum				1	1
119-Penicillium brasilianum		1			1
134-Penicillium adametzioides\spinulosum			1		1
totals					29

3.6 Endophytes in seeds

A background check was made to determine what, if any, endophytes were present in seeds from cultivars 38V12 and P0021 before being used in assays. Of the 28 isolates recovered directly from seed and sequenced for the ITS region, 22 were identified by BLAST/UNITE from 11 different genera (Table 3.8). Six isolates did not amplify in PCR and three isolates were undetermined at genus level.

Table 3.8 Endophyte species identified from seeds

Seed endophytes identified
Beauveria bassiana
Fusarium poae
F. proliferatum/Pestalotiopsis disseminata
Fusarium verticillioides
Microdochium oryzae/Phyllachora
Microdochium albescens/Phyllachora
Mucor hiemalis
Mucor racemosus
Penicillium raistrickii
Trichoderma viridescens

Chapter 4

Discussion

4.1 Main findings

This project aimed to identify beneficial endophytes from within maize plants growing in NZ. Mature maize plants from two regions in NZ, Waikato and Canterbury, were surface sterilised and fungi were isolated from the roots, stems and leaves. A large number of fungi were isolated with 322 isolates identified from 34 plants. Fungal isolates were then identified using morphological and molecular approaches. To determine which fungi might have potential as BCA's against pests and diseases, several bioassays were used. Firstly, dual culture methods were used, where putative endophytes were grown on the same media as a plant pathogen (*S. turcica*). The results from this testing and other assays (not reported here) led to a selection of 21 fungal isolates belonging to 13 genera to be tested in *in planta*. Seeds were coated using a polymer mix to establish in maize plants then challenged by an insect or a plant pathogen. Maize plants inoculated with putative endophyte isolates, from seed cultivar P0021, resulted in nine out of the 21 isolates tested were shown to be more resistant to disease and 13 isolate out of the 21 isolates in cultivar 38V12 were more effective when applied to two month old leaves. Leaves from plants similarly treated with the 21 isolates were fed to *H. armigera.* Seventeen fungal isolates treatments out of the 21 resulted in higher mortality than those feed untreated control plants and, in many cases, survivors had reduced weight gain. From these results, eight isolates were selected as having potential as a BCA against plant pathogens and or insect pests when added as a seed coating to maize.

4.2 Maize screening

The first objective of the study was to identify naturally occurring endophytes in maize. The screening of maize plants in NZ recovered a large number of isolates from both years (322 isolates). This is consistent with other studies sampling the whole plant of different plant species. For example: Bean (*Phaseolus vulgaris*) seeds planted in sterile conditions and grown for eight days had. 394 endophyte (from 42 taxa) isolates found from roots through to the top leaves with more endophytes present in seedling shoots than the roots (Parsa *et al.* 2016). In a study on rice (*Oryza sativa*), Zakaria *et al*. (2010) looked at 10 rice plants testing for endophytes from the roots, stems, leaves and the leaf sheath. They isolated 110 fungi from the four locations with a further 40 isolates recovered from seeds. Araújo *et al.* (2000) examined bacterial communities (mainly actinomycetes) and recovered 499 strains from maize roots (22 actinomycetes) and leaves (31 actinomycetes). A study by Patel *et al.* (2013) looked at both

monocots and dicots from a range of different plant species and identified 86 isolates from eight different genera. While the last study focused on different plant species from monocots and dicots, it does reinforce the number of microbes found in a single plant can be high. The diversity of fungal endophytes in coffee plants (*Coffea arabica*) from the entire plant recovered a large number of isolates (843) from four different countries with 87% coming from Ascomycota and only 13% coming from Basidiomycota (Vega *et al.* 2010). All the previously mentioned studies recovered large numbers of isolates from multiple taxa. This suggests it is 'normal' to have a large number of endophytes present in a plant at a given time. In this study on maize a large number of isolates were also found with 322 isolates recovered belonging to 73 different species. The majority of fungi recovered in this study came from Ascomycota represented by two main classes; Dothideomycetes and Sordariomycetes. Only 11 species did not come from Ascomycota. However, this may partly reflect the bias of the sampling method, as only isolates that grew on PDA were recovered.

In the current study, there were ten genera found in both regions of Canterbury and Waikato. The genera in common were; *Alternaria, Cladosporium, Drechslera, Epicoccum, Fusarium, Mucor, Phoma,* and *Trichoderma*. While *Penicillium* was found in both regions, at the species level they differed. Other genera in common in both geographical regions at species level were; *Alternaria alternata, A. arborescens, A. tenuissima, Cladosporium cladosporioides*, as well as six *Fusarium* species including *F. graminearum* and *F. oxysporum*. No published studies were found that compared differences in endophytes found in geographical areas in maize grown in NZ. Fisher *et al*. (1992) studied endophytes in maize in Devon, UK, and found seven species to be the same as in this study; six of these species were the same as their 11 identified. These were; *A. alternata, C. cladosporioides, F. graminearum, F. oxysporum, Trichoderma harzianum* and *Microdochium bolleyi.* They noted there was a large number of genera in agricultural crops but did not specify which genera. In another study by Fisher and Petrini (1992) of rice in UK, endophytes were recovered from the leaf blade, leaf sheath and roots. Six species were in common with this study out of a total of 31 species. In a study by Parsa *et al*. (2016) in Colombia they also found in beans seeds the same species of *F. oxysporum* and *C. cladosporioides, Chaetomium globosum* and *Epicoccum nigrum*. Their study isolated 394 fungal endophytes and, with the exception of one isolate (*Marasmius* aff. *nigrobrunneus*), all belonged to Ascomycota division. A study by Zakaria *et al*. (2010) from rice in Malaysia, recovered 110 isolates from five genera but there is no identification given of isolates at species level. However the genera they recorded as present, *Fusarium, Penicillium, Curvularia* and *Aspergillus,* are in common with this study. The genera found in this study appear to be a common occurrence regardless of host species or geographical location from the literature reviewed.

In a study by Patel *et al*. (2013), 86 isolates were found from 22 plants, some of the same fungal isolates belonging to the same genera as this study, *Aspergillus, Nigrospora, Mucor, Curvularia,*

Fusarium, Alternaria and *Stemphylium* from both monocot and dicot plants from the Jabalpur region, India. There is no mention of the recovered species in each genus, which means 86 isolates could have only been a few fungi recovered multiple times. It is suggestive that some species are common in studies throughout the world. These could represent opportunistic facultative endophytes, capable of invading plants but having other ecological lifestyles (Holder *et al*. 2007). For example when a plant is close to senesce, the fungi, such as *B. bassiana*, may grow to the exterior to sporulate and where it can attach and infect insects (Stone *et al.* 2007). Before the insects' death, the insect may have travelled some distance taking the fungus to new locations. Or is the fungus common due to isolation methods? It is known that some fungi cannot grow in the laboratory nor tolerate traditional culture methods. There would need to be further research to distinguish whether the common genera at species level is due to isolation methods or in-fact the species commonly occurs in different plant hosts and species. As mentioned in the previous paragraph some species do appear to be present more often regardless of host species or location.

4.3 Culture methods

In this study 75% of species have been identified as occurring in more than one maize plant. In the study of Casieri *et al.* (2009) 25% of the fungi isolated were found in more than one plant. Recovery levels of 25% of the species in more than 1 plant have also been mentioned in other studies (Fisher *et al*. 1992; Patel *et al.*2013; Parsa *et al.* 2016; Zakaria 2010). The current study used direct isolation culture methods for isolating fungi. Fungal cultures grew in such tight proximity on isolation plates, and that may have excluded some isolates (Pan and May 2009; Porras-Alfaro and Bayman 2011). Fungi have different growth rates and traits with more dominant fungi out competing slower growing fungi. For this reason plates were checked daily for the first two weeks then periodically for the next two months to check for any new fungal colonies. A final check at six months was made of samples kept in the fridge before samples were discarded. In addition, culture methods may favour the species identified, these may be more competitive and outcompete other fungi, or they may be more adapted to growing in laboratory conditions. Of course not all fungi can be cultured (Hyde and Soytong 2008). The technique used for this study included PDA with antibiotics and a liquid media (PDB) which may have favoured some fungi and excluded others. PDA with antibiotics was added to prevent bacterial growth outcompeting fungi. Initial isolation of fungi grew bacterial isolates that over grew the plates quickly excluding most fungi. Antibiotics inhibit the bacterial growth but may also inhibit some fungi (Fisher *et al*. 1992; Larone 1989). Another aspect found in this study was some isolates did not survive culturing for testing in assays. While an initial isolation obtained the isolates they were not able to be subcultured. It is unknown if it was the growing medium of PDA or other factors such as storage in low

temperatures. These were *Stemphylium vesicarium, Pyronema domesticum, Candida sake* and *Ascochyta pinodes.*

Niaz and Dawar (2009) looked at the fungi in seed-borne mycoflora in maize. Three different extraction methods were used, culture by agar plating, blotter and deep freezing. The blotter technique placed sterile seeds on three layers of moist blotter. For the deep freezing method, sterile seeds were placed in an incubator then frozen, then placed back in the incubator for a defined time and a specific temperature. They did find straight culturing onto media achieved more results and worked better for *Rhizopus, Aspergillus, Cladosporium* and *Curvularia.* Deep freezing worked better for *Drechslera, Fusarium* and *Penicillium,* suggesting isolation methods did play a role. Other methods of identification, excluding microscopy and sequencing, were not used in this study due to costs and time. It would be interesting to test maize tissue for endophytes by non-culture methods such as Next Generation Sequencing to examine how many other fungi may be present in maize.

4.4 Detection of endophyte in plant tissue

A selection of tissue samples were taken from the plant from the roots extending through to upper stem. This may have also influenced the endophytes recovered, as the plants were over a metre high and tissues were taken at random. The tissues tested represented less than 1% of the plant mass. For example in the lower stem location (Fig. 2.1) of the plant, samples were taken from three sites; top leaf, bottom leaf and stem. The length varied from 25 to 40 cm with approximately only 5 cm of tissue plated on PDA per site. The areas which included the leaf tissues measured more than 25 cm in length but again approximately only 5 cm of tissue was plated and likewise with the stem. Therefore it is probable not all fungal species are recovered.

There are few studies with maize sampling from the entire plant to compare. Fisher *et al*. (1992) focused on stems and leaf tissue in maize. Their study found the majority of the fungi recovered came from the centre or pith/core of the stem. They suggested there was tissue specificity at species level, e.g. *Fusarium* spp. and *Microdochium bolleyi* were found in the lower part of the stem whereas *A. alternata* was found in the leaves suggesting a leaf endophyte. However *Alternaria alternata* was found in all tissues sample sites in this study (Appendix A8) ruling out the possibility that *A. alternata* is solely a leaf endophyte.

Casieri *et al*. (2009) linked the xylem vessel size to the ability of the fungus to travel within the plant. This could explain the reduction in numbers towards the top of the maize plant. The height when these plants were sampled was 1-2 m high and towards the end of their natural life. This would also suggest

as the plant is getting closer to senescence the endophytes congregate towards and into the seed or externally to sporulate (Stone *et al.* 2017; Zakaria *et al.* 2010). Stone *et al*. (2017) also confirmed some endophytes do spend part of their lifecycle outside of the host plant in response to senescence. A pattern described as 'typical for latent pathogens' had fruiting bodies appear on leaves with senescence but spores were not released until bud opening or leaf emergence.

The above examples suggest species may target preferred areas for colonisation. Detection may depend on the species preference for tissue habitation of the host plant as well as culturing methods. The absence of these species may in fact be due to both the fungus' lifestyle traits and culture methods.

4.5 Colonisation rates

Colonisation of the host plant by endophytes seems to vary. It is known that some endophytes transmit vertically through infection internally of seed tissues and/or horizontally by growing externally from the plant such as *B. bassiana* infecting a neighbouring plant or insect (Carroll 1988; Stone *et al*. 2007). In a study by Bing and Lewis (1993), *B. bassiana* was applied as a foliar application at the whorl stage in maize and as an injection into the xylem of the plant tissue. The effect was only short term with the foliar application while the injection had a longer term effect colonising the plant. Inoculation method had an effect on the colonisation rates. Internally in the maize plant, the hyphae colonise between the cells and are also known to enter the xylem vessels (Vidal and Jaber 2015). It was therefore thought colonisation of the entire plant should occur. But in a study by Tefera and Vidal (2009) leaves of sorghum plants were inoculated with *B.bassiana* expecting the fungus to travel systemically through the entire plant but it was found colonisation did not occur in the roots when grown in 3 different mediums; non-sterile soils, sterile soils and vermiculite. Another study by Landa *et al.* (2013) found colonisation did not persist in inner tissues of poppy plants longer than 10-15 days after inoculation of *B. bassiana*. Rodriguez *et al*. (2009) looked at lifestyle traits of class II (belonging to Ascomycota or Basidiomycota) endophytes, mentioning *Fusarium* and *Curvularia* spp. as being present in the rhizosphere in low densities compared to *Phoma* sp.. *Phoma* sp. was found to be a common root endophyte and could confer fitness to the host plant. While this study was referring to the *Phoma* in the rhizosphere, it may be the same principle for other endophytic species. The suggested reasons for low and specific areas of colonisation in the above studies include not only the species of fungus specificity but environmental conditions the host is exposed to, as well as methodology used for inoculation and nutritional status of the plant. Reisolating the species used to inoculate the plants may not necessarily mean the isolate will or can become endophytic. In the *H. armigera* bioassay, tissue samples were taken from all parts of the plants being fed to the larvae to test for inoculated

endophytes. The results show low levels (less than 1%) of tissues were able to recover the applied fungus from tissues. This was similar to reports in Vidal and Jaber (2015). Their conclusion suggested the rhizosphere environment and inoculation methods of systemic growth of the inoculated isolate via roots affected colonisation rates. It may be endophytic but in this study they have been hard to prove having to sample large numbers to find the inoculated endophyte present. It may be that another more effective method is needed to establish they are present and the endophyte has not, in fact, died out or the possibility of no colonisation occurred. The plant tissue samples were all taken when the plants were over one month old. As stated by Vidal and Jaber (2015) the colonisation by the endophytes may not have persisted beyond two weeks or it may simply be like looking for a 'needle in a haystack'.

4.6 Inoculation method and site of inoculation

Vidal and Jaber (2015) looked at *Lecanicillium lecanii* in monocots and dicots and found high variability of colonisation between species including down to the plant cultivar level. In one plant a high colonisation of fungi was recorded and the next plant there was no colonisation. Studies they looked at reveal lower colonisation rates and persistence in inoculum applied to leaves compared to roots or seeds. If it is known as a leaf endophyte, does inoculation as a BCA have to be via the leaf? Can the effect still be the same if the inoculum is applied to the seed or by roots? In a study by Tefera and Vidal (2009) application of *B. bassiana* was by leaf, seed and soil inoculum. Sterile seeds were soaked in a conidial solution for 10 minutes and for the leaf application a spraying method was used. Seed inoculation in sterile soils, with conidia, was not shown to result in colonisation of the leaf but there was a high colonisation in the stem and roots. In seed inoculated plants in non-sterile soils there was no colonisation of the stem or leaf. Inoculation by leaf method resulted in no colonisation of the roots but a high colonisation of the leaf was recorded.

In a separate trial (data not shown here) maize plants were grown on water agar to V2 growth then a selection soaked in a conidial solution for 10 minutes. Plants were tested two hours after inoculation and confirmed the isolates were taken up by the plant. This however does not prove colonisation occurred just confirms the presence of the isolate was taken up internally. The method of inoculation for this study was by seed coating. Fisher *et al.* (1992) suggested *Alternaria alternata* was a leaf endophyte however as mentioned earlier *A. alternata* was also found in the roots in naturally occurring tissue sampling. This contradicts *A. alternata* as a leaf endophyte. This would need more testing to determine if it could be inoculated and recovered from the site of inoculation.

Variables in this study such as the environmental conditions and nutrient levels were the all the same for each plant (randomisation applied), ruling out some of the above reasons for low or no

colonisation. This leaves the question of the fungus species or the length of time since inoculation as a possible reason for low colonisation rates. The low levels of infection rates found when isolating back for proof of inoculated isolates may be due to the specificity of the species as an inoculum. The method and place of inoculation as well as fungal preferences may have had an influence on colonisation success and long term survival. A large number of agar plates (295) were tested to get a total of 29 endophytes confirmed belonging to 12 isolates. Seven isolates were not found in any plant tissues. However the assay results, when isolates were compared to controls, do suggest the isolates were present and do have an effect (Fig's. 3.14 and 3.18). It must be noted the sampling for the inoculated isolates was done at an older stage of plant growth (V4, approximately five week old plants grown in nursery conditions) so as to achieve sufficient plant growth for leaf material for feeding insects, and for the disease assay, applying the pathogen to V2 and V3 leaves. A total destruction of the plant was needed for testing roots and stems so for disease assay these plants were harvested on completion (V6 growth) with the tissue samples being taken throughout and on completion for the insect assays from extra plants grown at the same time. The isolating back of inoculated endophytes tested only 21 species of which over half (12 species) recovered (Table 3.7), from the original sampling of isolates recovered at the beginning of this study of over 100 identified (Appendix 3). It is therefore unknown how other endophytes, not tested, would behave.

4.7 Seed endophytes

Preliminary testing of the seeds (DuPont Pioneer® P0021 and 38V12) in this study for endophytes found seven genera, *Beauveria, Fusarium, Microdochium* or *Phyllachora*, *Mucor, Penicillium* and *Trichoderma* spp. already present in the seed before any testing (Chapter 3, section 3.6). Zakaria *et al*. (2010) isolated 40 fungal isolates from six different genera from rice seeds showing similar results to this study. Two isolates were identified as possibly being *Phyllachora* species. *Phyllachora* is thought not to be a seed borne endophyte (Chalkley *et al*. 2010). *Phyllachora maydis* has been identified as a pathogen commonly called tarspot found on grasses and cereals. However, this was a putative identification of a recovered isolate found when doing a background check of fungi already present in the seeds. This would need further work to establish if in-fact it is present and as a seed endophyte.

Two genera found in my study, *Fusarium* and *Curvularia,* were also noted as being seed endophytes by Zakaria *et al.* (2010). Logrieco and Moretti (1995) found *Fusarium proliferatum,* a known pathogen, in maize seeds in Italy. Crocker *et al*. (2016) found *Alternaria, Epicoccum* and *Fusarium* species present in ten selected species of wetland plant seeds. The last three genera mentioned were previously reported as plant pathogens and have been found overwintering in seeds and in the rhizosphere however there was no effect on seed germination (Appendix D1). Crocker *et al*. (2016) suggest some

pathogens persist in healthy seeds until the next seedling establishment and then affect seedlings when at their most vulnerable point of crop establishment. In a separate trial (data not shown) to look for a pathogen to use for insect and disease assays, an isolate *Fusarium graminearum* (isolate 9A), from this study was trialled. This seed treatment did not result in establishing any background disease despite some of the fungi belonging to known pathogenic species. This suggests that known pathogenic species may also have endophytic strains and in the conditions trialled was not a latent pathogen either. However it must be noted for this study when *F. graminearum* (isolate 9A) was tried as a pathogen against plants grown under laboratory conditions, and applying a stress such as water deficiency, the fungus had no effect at the time applied.

An observation from this study was the seed endophytes identified at the beginning of this study (2013) were not present in the seed lot at the end (2016) (data not shown). It appears the endophytes have most likely died out in that time frame.

DuPont Pioneer® has developed seed to be more resistant to the disease NLB. The two cultivars used, P0021 and 38V12, in these trials did not perform as expected. The resistance scores provided by the company stated P0021 had a higher score rating of 0.7 than 38V12 at 0.6 for disease resistance. The actual disease score rating when the pathogen was applied in laboratory was 0.89 for 38V12 and for P0021 cultivar disease level increased to 1.1. These figures are the averages from all the treatments (Appendix 1). These experiments were run in a controlled environment with a small replicate number compared to large paddocks in field trials which may influence results.

Crocker *et al.* (2016) isolated endophytes from a seed bank environment in America. The authors suggested the fungal colonies which come from seeds were known seed pathogens, naming *Pythium* sp. as an example. *Pythium* was found in low frequencies of isolation which they suggest may be due to *Pythium* having a poor competitive ability. This information could be transferred to the laboratory environment, showing as a poor competitor; therefore frequency of isolation was limited. This was in fact the case in this study where *Pythium* was found only once (isolate 141) and even then identification was as either *P. aristosporum or P. arrhenomanes*. It is therefore possible more species may have been present but were outcompeted by faster growing species. The isolation of isolates onto PDA plates showed a high number of species present. Competition may have led to the exclusion of some species favouring stronger genera such as *Fusarium*. This may explain the high number of *Fusarium* found compared to one *Pythium* sp. in this study.

4.8 Ecological roles; non-pathogenic and pathogenic

Some of the species recovered in this study have previously been reported as interacting with plants in a variety of roles. The studies on the fungi recovered from this study were examined for life history traits (Appendix D1); i.e. whether previously reported as being beneficial and/or pathogenic in maize. Not every species had relevant information. The plants used for the original isolations in this study were all healthy so the assumption was the isolates identified as 'pathogens' from known pathogenic species were actually latent pathogens (Carroll 1988; Fisher *et al.* 1992; Fisher and Petrini 1992; Maharachchikumbura *et al.* 2011). Latent pathogens can become disease causing if the plant is stressed by environmental changes, but the change may also be from a latent pathogen to a symbiont (Carroll 1988; Maharachchikumbura *et al.* 2011; Zakaria *et al.* 2010).The environmental change may be the seasonal occurrence of senescence in the plant. Zakaria *et al.* (2010) suggested the status of an endophyte may change due to a change in host defence in response to environment so the plant is the trigger for the endophytes' change in status.

Crocker *et al*. (2016) suggested some of the fungal isolates recovered in their study were non-pathogenic to plants. Virulence was tested by seedling germination and survival after inoculation with the fungi into three different host species. The fungi identified as non-pathogenic, the authors believed, still had an interaction (direct or indirect) with soil biota as well as seedling mortality. Both latent pathogen and non-pathogen strains can be influenced by the environment especially the soil nutrients and biota. The interactions become complicated as the host plant is equally affected by the environment which in turn may also influence the endophyte community.

It was interesting that several of the fungi recovered in this study represented species previously reported as plant pathogens, although they did not cause visible disease symptoms. As mentioned above, in a separate trial of *Fusarium* spp. as a potential disease causing fungi, several of the strains recovered showed no disease ability on maize, suggesting some species may have endophytic and pathogenic abilities but also some known pathogens may also have no virulence. In the current study some of the common fungi identified as belonging to previously described pathogen groups include; *Alternaria, Aspergillus, Drechslera, Fusarium, Gaeumannomyces* and *Pithomyces* (Abe *et al.* 2015; Brook 1962; Deacon 1975; Stone *et al.* 2017). A study by Casieri *et al*. (2009) referred to natural endophytes as being part of the host plants' ability to cope with environmental change but that fungal communities may be influence the balance between 'good' or protective endophytes and 'bad' being latent pathogens.

Stone *et al*. (2017) divided endophytes into ecological categories of lifestyle traits such as epiphytes, saprophytic, mutualistic or latent pathogens. An example mentioned was *Fusarium* sp. as a latent pathogen and *Alternaria* and *Cladosporium* as epiphytes, all are capable of internal colonisation as

endophytes. These three genera were commonly recovered from plants in my study. However in this study testing was for endophytes only. The surface sterilisation methods at the onset of maize testing resulted in an incomplete sterilisation and the isolation of a potential epiphyte, 43A *Mucor racemosus*, as this was isolated from a control plate where sterile stem tissue was rubbed on a PDA plate. Interestingly, this putative epiphyte performed strongly *in planta* testing against disease. *Mucor racemosus* was also recovered as an endophyte of leaves after seed treatment in my study (sterile techniques altered and control plates clear), suggesting both epiphyte and endophyte abilities. The surface sterilisation methods were altered increasing bleach times (five minutes) until control plates were clear with no growth after 3 days incubation. This method was used for all further surface sterilisation to overcome sterilising issues and was also suggested by Hyde and Soytong (2008) as a potential problem when isolating endophytes. Surprisingly endophytes were still recovered from silk and thin roots samples. It was expected the length of time would destroy these tissue samples but the number of endophytes found may have been reduced. Other methods had to be adjusted as the study progressed as not all methods worked for every isolate, e.g. media type-agar or broth (Hyde and Soytong 2008; Newcombe *et al.* 2016).

4.9 Secondary metabolites

Mycotoxins are produced by fungi such as *Fusarium*, *Penicillium* and *Aspergillus* species. These mycotoxins are a group of secondary metabolites produced by the endophyte which are involved in the production of chemicals such as fumonisins, zearalenone, aflatoxins, citrinin, penicillic acid in response to a stress like herbivory (Ismaiel and Papenbrock 2015). Some secondary metabolites can be harmful to humans, animals and plants. Mycotoxins can affect the seed, plant viability, growth and plant development. Several of the fungi known to produce secondary metabolites were found in my study such as *Fusarium, Cladosporium*, and *Alternaria.* These were described as 'field fungi' and access the seed during plant development while *Aspergillus* and *Penicillium* spp. were called 'storage fungi', causing spoilage once harvested (Ismaiel and Papenbrock 2015). One particular mycotoxin produced by *Fusarium* spp. that is of concern if fed to animals is fumonisins (Eckard *et al*. 2011). The harvest of the last samples was immediately prior to harvesting of maize for silage. These plants were numbers 14-17 sampled in 2014 (Appendix A4). Four different species of *Fusarium* were identified; *F. oxysporum, F. proliferatum, F. equiseti* and *F. konzum*.

F. verticillioides, F. culmorum and *F. graminearum* are three species identified as causative of Fusarium head blight and stalk and ear rot (Eckard *et al.* 2011; Logrieco and Moretti, 1995). Eckard *et al.* (2011) noted maize has a higher colonisation of *Fusarium* species than the smaller cereal crops, giving two examples of 13 and 15 different *Fusarium* species found in Swiss maize in 2005 and 2006 respectively.

This current study identified 13 *Fusarium* species with possibly a further eight species identified as one of two species e.g. *Fusarium lateritium/acuminatum*. A total of seven of the *Fusarium* species identified produce mycotoxins previously listed from other studies such as*; F. avenaceum, F. culmorum, F. equiseti, F. oxysporum, F. proliferatum* and *F. verticillioides.* These were collected from 91% of the plant tissue samples in 2014 and 72% in 2015. However as mentioned earlier, this represents less than 1% of the plant mass tested so it is very possible there was more species present and possibly every plant contained one *Fusarium* species. *Fusarium* species also appear to be more prevalent in plants that were senescing. This would make sense if saprophytic fungi found in higher numbers in both plants and soil, as shown by Fravel *et al*. (2002). Eckard *et al.* (2011) confirmed maize had a higher number of different species present compared to small grain cereal crops supporting the finding of a high number of *Fusarium* spp. found in this study.

The presence of mycotoxins and other secondary metabolites could be useful in BCAs as these traits occur naturally and set themselves apart from synthetic pesticides (Murphy *et al*. 2015). The secondary metabolites produced by isolates in dual culture challenge were responding to only one pathogen on a selected medium. This does not always represent the same behaviour in the plant when put in the field environment (Butt and Copping 2000). In the field environment conditions cannot be controlled. Dual culture methods were used to screen the large number of isolates identified for testing against insect and disease bioassays to reduce the number of isolates to a more manageable number to test in the time frame given, but may not be indicative of endophytic usefulness.

4.10 Endophytes as BCA's

A good BCA needs to be virulent against pests and/or diseases maintaining them below the economic threshold and it needs to work quickly to prevent large scale spread (Butt and Walden 2000; Vidal and Jaber 2015). Application and formulation, as well as ease of use, need to be considered and the product must be able to be mass produced for commercial use. From a commercial viewpoint, regulators and researchers need to know the level of mycotoxins in and mode of action of the potential BCAs, the agent must be cost effective and to consistently colonise the host plant, in other words it needs to be easily reproducible (Bing and Lewis 1993; Vidal and Jaber 2015). Having an endophyte as a BCA is a good alternative to chemicals and is generally more accepted by the consumer than a genetically modified organism (GMO) (Bing and Lewis 1993; Murphy *et al.* 2015). One of the benefits of using an endophyte as a BCA is it is already known to colonise plants and may protect the plant against both disease and insects (Backman and Sikora 2008; Bing and Lewis 1993; Ownley *et al*. 2004). It would be beneficial if an endophyte as a BCA could give an 'inbuilt immunity' to the host plant

and can increase the plants ability to cope (Backman and Sikora 2008; Butt and Walden 2000; Chen *et al*. 2016a).

Some fungi (including non-endophyte applications-e.g. sprays) are already in use or trialled as BCAs against disease include non-pathogenic *F. oxysporum* against other pathogenic *F. oxysporum* species and *Trichoderma harzianum* against several diseases (Butt and Copping 2000; Fravel *et al.* 2003). BCAs already developed for commercial use for control of insects include; *B. bassiana, Lecanicillium lecanii* (also known as *Verticillium lecanii*), *Metarhizium* sp. and *Paecilomyces* sp. against multiple insects (Butt and Copping 2000; Vidal and Jaber 2015). A few microbes are still being developed for use against weeds, such as *Alternaria, Colletotrichum* and *Phytophthora* species (Butt and Copping 2000). In a study by Chen *et al.* (2016), development of a fungal BCA for phytopathogens includes *Trichoderma gamsii* against fungi causing root rot, in *Panax notoginseng*. *P. notoginseng* is a traditional Chinese medicinal herb affected by multiple fungi causing root rot disease. The effect from root rot can cause severe crop damage and crop losses. A natural control such as *Trichoderma* is seen as an environmental friendly solution.

For this study the aim was to find fungi that may have the potential to act against insects and/or plant pathogens when applied to seeds, and establishing endophytically. It is possible these fungi have other uses not investigated as discovered by then literature review of isolates in Appendix D1. A number of genera recovered do have previous studies showing a diverse range of benefits, like biotechnological use as producers of enzymes for industrial uses such as amylases (*Aspergillus* sp.), lipase production (*Cladosporium* and *Penicillium* spp.) and cellulases (*Cladosporium, Aspergillus* and *Penicillium* spp.) and proteases (*Mucor circinelloides*) (Abe *et al.* 2015; Ismaiel and Papenbrock 2015; Santiago and Motta 2008). Cellulases are used in food production, pulp extraction for vegetable and fruit juices, as well as textile industry, brewery and ethanol production such as fuel. Cellulases are also available commercially from *Trichoderma* and *Aspergillus* spp. Other genera like *Rhizopu*s, is used in biodiesel (Ban *et al.* 2012) and *Penicillium purpurogenum* is known for biosorption of heavy metals such as cadmium, lead, mercury and arsenic (Say *et al.* 2003). The previous examples show the flexibility of species found in this study can have multiple end uses.

Some of the isolates identified in this study have been reviewed as being capable of dual life stages and can be both beneficial and pathogenic. Some of the beneficial endophytes found in this study have been used or are currently still in use commercially such as *Beauveria* and *Bionectria* spp. have been used on plants as sprays against insects such as European corn borer (*Ostrinia nubilalis*), Colorado potato beetle (*Leptinotarsa decemlineata*) and NZ grass grub (*Costelytra zealandica*) on host plants in maize and artichoke (*Cynara scolymus*) (Bourner *et al.* 1996; Guesmi-Jouini *et al.* 2014; Ownley *et al.* 2008; Wagner and Lewis 2000). *B. bassiana* was used in seed treatment to control *Rhizoctonia solani,*

damping off disease in tomatoes (Ownley *et al.* 2008). *Trichoderma* has been widely used as a growth promoter and as a commercial inoculum, e.g. a *Trichoderma* bio-inoculant-ArborGuard™ is used to protect pine (*Pinus radiata*) seedlings from disease and boost plant growth (Hill *et al.* 2010; Maag *et al.* 2013). Endophytes affecting insects can also include entomopathogenic fungi such as *B. bassiana*, *Bionectria* spp. as mentioned above and *L. lecanii,* putative endophytes found in this study. Research suggests the insects can be affected when feeding on plants colonised by these species however previous studies results were highly variable (Vidal and Jaber 2015). Mycosis of insects is rarely reported when these fungi are presented as endophytes. This study noticed an effect from plants eating endophytic leaves compared to controls (Tables 3.5 and 3.6). *Beauveria* and *Lecanicillium* mortality was low (3 and 2 respectively) in comparison to *Rhizopus* and *Fusarium* both having 8 deaths recorded. Clearly more research is needed to determine if the mortality in insects is due to the fungus directly, the metabolites produced by the fungus, or changes in the plant that affect palatability and food quality.

Vidal and Jaber (2015) outline several areas of concern for BCA inoculation as endophytes;

1. endophyte host plant specificity,
2. method of inoculation,
3. mode of action-direct or indirect on insect,
4. variability in colonisation,
5. persistence of effect in the field or even glasshouse under variable environmental conditions and nutrient availability,
6. inter-specific interactions from other endophytes influencing colonisation, and
7. higher rates of colonisation were present in sterile soils.

Other lesser known reported BCA species found in a study have been recorded previously: *Penicillium adametzioides* acting against *Aspergillus* sp. in grapes, *Gaeumannomyces graminis* was controlled by *Phialophora radicicola,* and *Sporobolus* spp., an Australian weedy grass, can be controlled by *Nigrospora oryzae* (Ismaiel and Papenbrock 2015). One species isolated from maize identified was *Microdochium bolleyi* also known as *Idriella bolleyi*, previously reported as a BCA agent against cereal pathogens, in particular take-all patch disease (Lascaris and Deacon 1994). While the fungus was applied to the seed, the study looked at the nutrient requirements needed to be grown as a suitable BCA. Results found conidiation varied and depended on the nutrients in the media. In this study some isolates did not conidiate despite adjustments of temperature, humidity and media. If the endophyte was unable to produce conidia it was not going to be easily reproducible as a potential BCA and therefore these isolates were deemed not suitable and did not make the list of 21 isolates for further testing.

4.11 Screening challenges

Three isolates present could be of questionable origin. *Malassezia* sp. is known to cause a skin disease (Heitman 2011), *Penicillium* spp. can be a surface contaminant (Fisher *et al.* 1992), and *Pithomyces chartarum* is known as a causative agent of facial eczema in sheep. In this case the techniques were of such that the origin was from inside of the plant and all samples were carefully surface sterilised, but it is noted that contaminations are possible.

Dematiaceous fungi or dark pigmented isolates identified such as *Epicoccum* and *Trichocladium* species were difficult to identify (Larone 1989; Seidl 2010). The pigment interfered with the Chelex extraction or PCR amplification and extraction had to be repeated. Identification of *Epicoccum* was achieved by growing in PDB and taking a sample of mycelium at 2-3 days old then continued with Chelex DNA extraction and PCR.

The maize growing season is relatively short with ten plants from Waikato arriving at the same time. The processing of a large number of maize plants found fungi grew on the plants kept in the fridge within one week and these therefore had to be discarded. Zakaria *et al*. (2010) processed all tissue samples within 48 hours to avoid desiccation of plant tissue. This could be an indication of a change in community after harvest.

Ideally it would have been best to sequence every isolate by molecular techniques from every location from the plant sampled. However due to the expense of sequencing this could not be done. Once it was found large numbers of isolates were present, isolates were identified by morphology first so a representative could be sent for sequencing. Patel *et al.* (2013) used similar methods of identifying isolates by microscopic and morphology combined with literature and sequencing.

4.12 Summary

In this study a large number of fungi from 37 genera from maize from two regions, Canterbury and Waikato, NZ were isolated. Isolates recovered were identified, screened on mass in a dual culture assay to narrow down isolates numbers for a more manageable number for further testing. The selected isolates were tested *in planta* against a plant pathogen and a leaf-feeding insect pest. Eight fungi (Table 4.1) were found that showed promise as seed applied BCAs and will be further investigated. Four isolates stood out as the top performing isolates in all three criteria (*In planta, H. armigera* and endophyte recovery). These were *Sordaria fimicola*, *Mucor racemosus, F. acuminatum* and *F. proliferatum*.

Table 4.1 The 8 isolates selected for testing for potential as BCA

	Isolate number and name
1	36-*Sordaria fimicola*
2	43A-*Mucor racemosus*
3	95-8-*Trichoderma atroviride*
4	119-*Penicillium brasilianum*
5	129B-*Fusarium equiseti*
6	19-*Mucor fragilis*
7	53a-*Fusarium acuminatum*
8	51-*Fusarium proliferatum*

The origin of *Mucor racemosus* serves as a reminder not to discount any fungi as they may be of value as a BCA. It was unexpected to find such a large number of fungi present in maize and is worth remembering when working with other host plants. Identification methods continue to develop adding to the number of endophytes found in host plants especially with molecular techniques becoming available.

Appendix A

Screening for isolates and identification

A.1 Isolates identified through sequencing of the ITS regions of rDNA in 2014

DNA sample number	Region	Plant number	Location on plant	Specific isolation site	Genus & species identified (Synonym)
1	W	1	roots	radicle	*Fusarium culmorum*
2	W	2	roots	seminal	*Mucor hiemalis*
3	W	5	roots	brace	*Rhizopus oryzae*
4	W	6	ear	leaf husk	*Alternaria alternata*
5	W	2	lower stem	bottom leaf	*Alternaria alternata*
6	W	5	lower stem	bottom leaf	*Ascochyta pinodes/fabae*
7	C	7	roots	radicle	*Fusarium oxysporum*
8c	C	11	ear	leaf husk	*Fusarium culmorum*
9A	C	10	lower stem	top leaf	*Fusarium graminearum*
10	C	7	ear	kernel	*Mucor hiemalis*
11b	C	9	lower stem	top leaf	*Phoma glomerata*
12b	C	9	lower stem	top leaf	*Alternaria alternata*
12	C	9	lower stem	top leaf	*Alternaria tenuissima*
13	W	1	roots	radicle	*Bionectria ochroleuca*
14	C	10	upper stem	tassel	*Fusarium avenaceum*
15	C	11	upper stem	tassel	*Mucor hiemalis*
16	W	4	lower stem	bottom leaf	*Phoma pinodella*
17	C	7	ear	silk	*Pyronema domesticum*
J18	C	7	ear	leaf husk	*Beauveria bassiana*
19	W	5	ear	leaf husk	*Mucor fragilis*
20	C	9	roots	radicle	*Fusarium equiseti*
20	C	9	roots	radicle	*Fusarium avenaceum*
J21	C	7	ear	leaf husk	*Beauveria bassiana*
22	W	4	upper stem	top leaf	*Alternaria alternata*
23	W	3	lower stem	top leaf	*Epicoccum nigrum*
24	W	12	upper stem	stem	*Epicoccum nigrum*
25	W	6	ear	leaf husk	*Epicoccum nigrum*
26	W	5	ear	leaf husk	*Epicoccum nigrum*
27	W	6	roots	radicle	*Fusarium equiseti*
28	W	2	lower stem	bottom leaf	*Epicoccum nigrum*
29	W	6	ear	leaf husk	*Epicoccum nigrum*
30	C	10	roots	brace	*Fusarium avenaceum/acuminatum*
31	W	4	roots	seminal	*Fusarium oxysporum*
32a	W	12	lower stem	top leaf	*Fusarium verticillioides*
32ao	W	12	lower stem	top leaf	*Fusarium proliferatum/disseminata*
32Aw	w	12	lower stem	top leaf	*Fusarium sacchari/verticillioides*
32c	W	12	lower stem	top leaf	*Fusarium equiseti*

33	W	12	ear	kernel	*Fusarium oxysporum*
34	W	3	lower stem	top leaf	*Epicoccum nigrum*
35	C	7	upper stem	stem	*Fusarium proliferatum*
36	W	12	ear	silk	*Sordaria fimicola*
37	W	12	ear	peduncle	*Fusarium konzum*
37b	W	12	ear	peduncle	*Fusarium succisae/proliferatum*
38dark	W	4	ear	leaf husk	*Alternaria alternata*
38light	W	4	ear	leaf husk	*Alternaria consortiale (Ulocladium consortiale)*
39	C	9	ear	leaf husk	*Alternaria brassicae*
40b	C	7	ear	silk	*Stemphylium vesicarium*
41	W	4	ear	leaf husk	*Alternaria alternata*
42	C	9	upper stem rubbed	tassel	*Botrytis fabae/cinerea*
43	C	9	stem	epiphyte-c	*Mucor racemosus*
44	C	10	ear	leaf husk	*Fusarium oxysporum*
45	C	10	lower stem	top leaf	*Microdochium bolleyi*
45c	W	12	roots	brace	*Fusarium oxysporum*
45d	W	12	roots	brace	*Fusarium verticillioides*
45do	W	12	roots	brace	*Fusarium verticillioides/sacchari*
45ds	W	12	roots	brace	*Fusarium proliferatum*
46t	W	12	roots	radicle	*Trichoderma koningiopsis/gamsii*
47	C	8	lower stem	top leaf	*Paraphaeosphaeria michotii* (*Leptosphaeria michottii*)
48	W	1	roots	seminal	*Bionectria ochroleuca*
49	C	10	ear	silk	*Schizothecium fimbriatum*
50	W	2	ear	leaf husk	*Curvularia trifolii*
51	W	12	ear	peduncle	*Fusarium proliferatum*
52	C	7	roots	brace	*Fusarium equiseti*
53a	C	10	upper stem	stem	*Fusarium acuminatum*
54a	C	7	ear	silk	*Pyronema domesticum*
55	C	10	roots	brace	*Trichoderma hamatum*
56	W	5	roots	seminal	*Fusarium equiseti/incarnatum*
57	C	10	upper stem	stem	*Fusarium avenaceum*
58	C	10	upper stem	stem	*Fusarium avenaceum*
59	W	12	ear	peduncle	*Fusarium verticillioides/sterilihyphosum*
60a	C	10	upper stem	stem	*Fusarium avenaceum*
61	C	9	ear	leaf husk	*Candida sake*
62	C	11	lower stem	top leaf	*Alternaria infectoria*
63	W	1	roots	radicle	*Cladosporium colocasiae*
64	W	1	roots	radicle	*Alternaria alternata*
65	C	10	ear	leaf husk	*Alternaria alternata/tenuissima*
66	W	4	ear	leaf husk	*Alternaria tenuissima*
67	W	12	upper stem	top leaf	*Alternaria alternata*
68	W	5	lower stem	bottom leaf	*Ascochyta pinodes*
69	W	3	ear	leaf husk	*Alternaria alternata*
71	W	12	lower stem	top leaf	*Fusarium sterilihyphosum*
72	C	10	roots	brace	*Fusarium flocciferum*
73	C	10	upper stem	stem	*Fusarium avenaceum*
74	W	5	roots	seminal	*Fusarium equiseti*

75	W	5	roots	seminal	*Fusarium equiseti*
76	W	12	ear	peduncle	*Fusarium tricinctum*
77	W	1	roots	radicle	*Fusarium graminearum*
78	W	6	upper stem	tassel	*Curvularia trifolii*
79	W	6	upper stem	tassel	*Curvularia trifolii*
81	W	6	ear	silk	*Penicillium glabrum/adametziodes/spinulosum*
82a	C	10	upper stem	stem	*Fusarium avenaceum*
84	C	9	ear	leaf husk	*Alternaria infectoria*
85	C	11	roots	seminal	*Trichoderma koningiopsis/atroviride*
86	W	2	ear	kernel	*Lecanicillium lecanii*
87	C	9	-	-	*Fusarium proliferatum/Pestalotiopsis disseminata*
88	C	10	roots	brace	*Fusarium lunulosporum*
89	W	1	roots	radicle	*Fusarium tricinctum*
90	W	12	ear	kernel	*Fusarium verticillioides*
91	C	9	ear	leaf husk	*Alternaria infectoria*
92a	C	10	lower stem	top leaf	*Alternaria infectoria*
92b	C	11	lower stem	top leaf	*Alternaria infectoria*
93	C	8	upper stem	tassel	*Epicoccum nigrum*
94	W	6	upper stem	tassel	*Curvularia trifolii*
95-1	C	9	ear	leaf husk	*Trichoderma koningiopsis/gamsii*
95-2	W	6	roots	radicle	*Trichoderma koningiopsis/gamsii*
95-3	W	1	roots	radicle	*Trichoderma* sp.
95-4	C	11	roots	seminal	*Trichoderma hamatum*
95-5	C	11	roots	seminal	*Trichoderma hamatum*
95-6	C	11	roots	seminal	*Trichoderma hamatum*
95-7	W	12	upper stem	stem	*Trichoderma harzianum*
95-8	W	5	ear	leaf husk	*Trichoderma atroviride*
95-9	W	12	upper stem	stem	*Trichoderma koningiopsis*
95-10	C	8	roots	radicle	*Trichoderma asperellum*
96	W	2	ear	leaf husk	*Epicoccum nigrum*
97	W	6	ear	silk	*Penicillium citreonigrum*
99	W	12	upper stem	top leaf	*Epicoccum nigrum*
100	C	18	ear	silk	*Fusarium proliferatum*
101A	C	16	upper stem	tassel	*Fusarium sambucinum/venenatum*
102	C	15	upper stem	top leaf	*Phoma herbarum*
103	W	12	roots	brace	*Fusarium proliferatum/sacchari*
104	C	16	ear	kernel	*Phoma herbarum*
105	C	16	lower stem	top leaf	*Phoma herbarum*
106	C	16	ear	leaf husk	*Microdochium bolleyi*
107	C	15	ear	silk	*Phoma herbarum*
108	C	17	roots	brace	*Penicillium purpurogenum*
110	W	12	upper stem	stem	*Fusarium proliferatum/verticillioides*
111	C	15	roots	radicle	*Microdochium bolleyi*
112	C	14	roots	radicle	*Fusarium proliferatum*
112	C	14	roots	radicle	*Pestalotiopsis disseminata*
113	W	6	ear	leaf husk	*Alternaria alternata*
114	C	17	upper stem	stem	*Fusarium oxysporum*

115	C	16	ear	silk	*Aspergillus ochraceus*
116	C	19	roots	radicle	*Fusarium verticilliodes/proliferatum*
117	C	22	ear	kernel	*Botrytis cinerea*
118	C	19	upper stem	stem	*Mucor fragilis*
119	W	6	roots	brace	*Penicillium brasilianum*
120	C	16	roots	brace	*Fusarium cerealis/culmorum*
121	C	19	roots	brace	*Mucor racemosus*
122	C	19	roots	radicle	*Fusarium oxysporum*
123	C	21	upper stem	stem	*Fusarium equiseti*
124	C	9	ear	leaf husk	*Stemphylium globuliferum*
125	C	15	ear	silk	*Aspergillus ochraceus*
126	C	16	roots	brace	*Microdochium bolleyi*
127	C	20	ear	silk	*Didymella* sp.
128	C	14	lower stem	top leaf	*Microdochium bolleyi*
129	C	18	roots	brace	*Fusarium equiseti*
130	C	20	upper stem	stem	*Botrytis cinerea*
131	C	22	ear	kernel	*Botrytis fabae/cinerea*
132	W	5	lower stem	stem	*Cochliobolus intermedius/Curvularia trifolii*
133	C	19	roots	seminal	*Fusarium oxysporum*
134	C	7	ear	leaf husk	*Penicillium adametziodes/spinulosum*
135	C	16	roots	brace	*Sarocladium zeae* (*Acremonium zeae*)
137	C	19	ear	silk	*Wallemia sebi*
138	C	20	upper stem	tassel	*Malassezia restricta*
139	C	15	ear	silk	*Fusarium oxysporum*
140	C	20	upper stem	top leaf	*Alternaria infectoria/rosae*
141	C	21	ear	leaf husk	*Pythium aristosporum/arrhenomanes*
142	C	9	roots	radicle	*Fusarium avenaceum*
143	C	15	upper stem	stem	*Trichoderma koningiopsis/atroviride*
144	C	15	ear	silk	*Fusarium oxysporum*
145	W	5	roots	seminal	*Fusarium oxysporum*
146	W	4	roots	brace	*Fusarium oxysporum*
147	W	4	roots	seminal	unknown
148	C	20	upper stem	tassel	*Alternaria infectoria*
149	C	21	lower stem	stem	*Fusarium* sp.
150	C	21	ear	leaf husk	*Fusarium* sp.
151	C	10	lower stem	top leaf	*Fusarium verticillioides/sambucinum*
152	C	9	ear	leaf husk	*Stemphylium globuliferum*
153	W	5	lower stem	stem	*Ascochyta pinodes*
154	C	15	ear	silk	*Fusarium* sp.
157	C	10	roots	brace	*Fusarium flocciferum*

A.2 Isolates identified through sequencing of the ITS regions of rDNA 2015 season

Isolate	Region	Plant number	Location on plant	Specific isolation site	Genus and species identified (Synonym)
15-1	W	3	ear	kernel	*Alternaria arborescens*
15-2	W	3	upper stem	top leaf	*Alternaria alternata*
15-3	C	8	lower stem	bottom leaf	unknown
15-4	C	8	upper stem	bottom leaf	*Alternaria arborescens*
15-5	W	4	roots	seminal	*Gaeumannomyces* sp.
15-6	W	1	lower stem	top leaf	*Alternaria arborescens*
15-7	W	4	upper stem	bottom leaf	*Drechslera dematioidea*
15-7B	W	4	upper stem	bottom leaf	*Mucor circinelloides*
15-8	C	12	lower stem	top leaf	*Trichocladium* sp.
15-8B	C	12	lower stem	top leaf	*Chaetomium/Trichocladium*
15-9	C	10	lower stem	top leaf	*Alternaria arborescens*
15-10	C	9	upper stem	top leaf	*Alternaria arborescens*
15-11	C	8	upper stem	bottom leaf	*Alternaria arborescens*
15-11B	C	8	upper stem	bottom leaf	*Alternaria tenuissima*
15-12	C	11	roots	seminal	*Alternaria chartarum (Ulocladium chartarum)*
15-13	C	11	upper stem	top leaf	*Alternaria arborescens*
15-14	C	10	ear	silk	*Alternaria alternata/arborescens*
15-15	C	8	upper stem	bottom leaf	*Alternaria infectoria*
15-16	W	1	lower stem	bottom leaf	*Pithomyces chartarum*
15-17	C	11	lower stem	bottom leaf	*Drechslera dematioidea*
15-18	W	4	roots	seminal	*Gaeumannomyces radicicola*
15-19	W	4	upper stem	top leaf	*Nigrospora oryzae*
15-19B	W	4	upper stem	top leaf	*Mucor circinelloides*
15-20	W	2	upper stem	bottom leaf	*Drechslera dematioidea*
15-21	C	12	lower stem	top leaf	*Microdochium bolleyi*
15-22	W	2	roots	brace	*Alternaria alternata*
15-23	W	4	upper stem	bottom leaf	*Drechslera dematioidea*
15-24	C	11	lower stem	bottom leaf	*Alternaria arborescens*
15-25	C	8	ear	peduncle	unknown
15-26	W	4	upper stem	bottom leaf	*Pithomyces chartarum*
15-27	C	11	lower stem	bottom leaf	*Fusarium avenaceum*
15-28	C	12	upper stem	stem	*Alternaria infectoria*
15-29	W	3	roots	seminal	*Fusarium oxysporum*
15-30	W	4	upper stem	top leaf	*Nigrospora oryzae*
15-31	C	12	lower stem	bottom leaf	*Fusarium equiseti*
15-31B	C	12	lower stem	top leaf	*Botrytis cinerea*
15-32	W	5	roots	brace	*Fusarium proliferatum/oxysporum/verticillioides*
15-33	W	2	upper stem	bottom leaf	*Fusarium graminearum*
15-34	W	1	roots	brace	*Fusarium proliferatum*
15-35	W	4	roots	brace	*Fusarium graminearum*
15-36	W	1	roots	brace	*Fusarium oxysporum*

15-37	W	3	roots	brace	*Fusarium oxysporum*
15-38	W	2	ear	peduncle	*Fusarium oxysporum*
15-39	W	2	lower stem	top leaf	*Fusarium equiseti*
15-40	W	5	upper stem	stem	*Fusarium oxysporum*
15-41	C	10	roots	radicle	*Fusarium proliferatum*
15-42	W	4	roots	seminal	*Harpophora zeicola*
15-42B	W	4	roots	seminal	*Harpophora zeicola*
15-43	W	2	ear	leaf husk	*Trichoderma gamsii*
15-44	W	3	roots	radicle	*Penicillium* sp.
15-45	W	2	ear	leaf husk	unknown
15-46	W	3	ear	leaf husk	*Cladosporium tenuissimum*
15-47	W	1	upper stem	bottom leaf	*Cladosporium* sp.
15-48	W	3	roots	seminal	*Fusarium oxysporum*
15-49	C	9	ear	leaf husk	unknown
15-50	C	9	upper stem	bottom leaf	*Alternaria infectoria*
15-51	C	11	lower stem	top leaf	*Fusarium oxysporum*
15-52	C	11	lower stem	top leaf	*Fusarium oxysporum*
15-53	C	6	roots	seminal	*Chaetomium globosum*
15-54	C	10	lower stem	top leaf	unknown
15-55	C	12	roots	brace	*Fusarium proliferatum*
15-56	W	3	roots	seminal	*Fusarium oxysporum*
15-57	C	6	roots	brace	unknown
15-58	C	11	upper stem	top leaf	*Alternaria alternata*
15-59	C	10	roots	radicle	*Botrytis cinerea*
15-59R	C	10	roots	radicle	*Botrytis cinerea*
15-60	C	8	upper stem	bottom leaf	*Alternaria infectoria*
15-61	W	3	ear	peduncle	*Mucor circinelloides*
15-62	W	1	roots	radicle	*Rhizopus oryzae*
15-63	W	2	ear	silk	unknown
15-64	C	6	roots	seminal	unknown
15-65	C	6	roots	seminal	unknown
15-66	C	6	roots	seminal	*Epicoccum nigrum*
15-67	W	1	roots	brace	*Fusarium oxysporum*
15-68	C	9	roots	radicle	*Mucor hiemalis*
15-69	C	9	roots	radicle	*Mucor hiemalis*
15-70	C	12	roots	radicle	unknown
15-71	W	2	upper stem	bottom leaf	*Fusarium avenaceum*
15-72	W	4	roots	radicle	*Fusarium oxysporum*
15-73	C	6	upper stem	top leaf	unknown
15-74	W	4	ear	silk	unknown
15-74B	W	4	ear	silk	*Trichoderma gamsii*
15-75	C	6	roots	seminal	*Penicillium spinulosum*
15-76	C	11	ear	leaf husk	*Penicillium adametzioides*
15-77	W	3	upper stem	bottom leaf	*Phoma* sp.
15-77B	W	3	upper stem	bottom leaf	*Epicoccum nigrum*
15-78	W	5	ear	silk	*Cladosporium cladosporioides*
15-79	W	4	lower stem	bottom leaf	*Phoma paspali*
15-80	W	3	upper stem	bottom leaf	*Fusarium graminearum*

15-80B	W	3	upper stem	bottom leaf	*Fusarium equiseti*
15-81	W	4	roots	radicle	*Fusarium oxysporum*
15-81B	W	4	roots	radicle	*Trichoderma harzianum*
15-82	W	1	lower stem	top leaf	*Fusarium oxysporum*
15-82B	W	1	lower stem	top leaf	*Rhizopus oryzae*
15-83	C	9	upper stem	bottom leaf	*Alternaria alternata*
15-84	W	1	roots	brace	*Fusarium oxysporum*
15-85	C	8	lower stem	bottom leaf	*Alternaria alternata*
15-86	W	3	upper stem	tassel	*Alternaria infectoria*
15-87	C	9	roots	radicle	*Fusarium avenaceum*
15-88	C	9	roots	radicle	*Fusarium* sp.
15-89	C	11	lower stem	bottom leaf	*Stemphylium vesicarium*
15-90	C	9	roots	brace	*Fusarium equiseti*
15-91	C	10	roots	radicle	*Drechslera* sp.
15-92	C	8	lower stem	top leaf	*Alternaria alternata*
15-93	C	8	lower stem	bottom leaf	*Penicillium griseofulvum*
15-94	W	5	ear	silk	unknown
15-95	C	8	ear	peduncle	*Cladosporium* sp.
15-96	C	10	roots	radicle	*Fusarium avenaceum*
15-97	C	12	roots	seminal	*Fusarium avenaceum*
15-98	C	10	roots	radicle	*Drechslera* sp.
15-99	C	10	roots	radicle	*Fusarium graminearum*
15-100	W	2	upper stem	top leaf	*Alternaria alternata*
15-101	C	10	roots	seminal	*Fusarium avenaceum*
15-102	C	6	roots	brace	unknown
15-103	C	10	roots	radicle	*Drechslera* sp.
15-104	C	9	roots	radicle	*Fusarium avenaceum*
15-105	C	7	upper stem	top leaf	*Fusarium graminearum*
15-106	C	8	lower stem	bottom leaf	*Alternaria alternata*
15-107	W	1	roots	seminal	*Alternaria alternata*
15-108	C	12	roots	seminal	*Fusarium oxysporum*
15-109	C	10	roots	seminal	*Fusarium avenaceum*
15-110	C	11	lower stem	bottom leaf	unknown
15-111	W	2	upper stem	top leaf	unknown
15-112	C	9	ear	silk	unknown
15-113	C	9	ear	leaf husk	unknown
15-114	W	4	roots	brace	unknown
15-115	C	12	lower stem	top leaf	unknown
15-116	C	7	lower stem	bottom leaf	*Alternaria brassicae/alternata*
15-117	W	3	lower stem	top leaf	*Alternaria arborescens*
15-118	W	1	upper stem	top leaf	*Cladosporium cladosporioides*
15-119	C	10	roots	seminal	*Alternaria arborescens*
15-120	C	8	upper stem	bottom leaf	*Alternaria infectoria*
15-121	C	10	roots	seminal	*Drechslera* sp.
15-122	C	9	ear	leaf husk	*Cladosporium sinuosum*
15-123	W	1	upper stem	top leaf	*Cladosporium* sp.
15-124	W	4	upper stem	bottom leaf	*Penicillium chrysogenum*
15-125	W	5	ear	silk	*Cladosporium* sp.

15-126	W	4	upper stem	bottom leaf	unknown
15-128	W	2	upper stem	top leaf	*Fusarium graminearum*
15-129	C	10	lower stem	top leaf	*Malassezia restricta*
15-130	W	4	upper stem	top leaf	*Penicillium* sp.
15-131	C	8	ear	silk	*Paraphaeosphaeria sporulosa*
15-132	C	10	roots	radicle	*Botrytis cinerea*
15-133	C	6	lower stem	top leaf	*Fusarium equiseti*
15-134	C	9	roots	brace	*Cladosporium allicinum/herbarum*
15-135	C	12	lower stem	bottom leaf	*Botrytis cinerea*
15-136	C	8	ear	peduncle	*Sordaria fimicola*
15-137	C	12	roots	radicle	*Trichoderma asperellum*
15-138	C	12	roots	radicle	*Trichoderma asperellum*

A.3 Summary of isolations from 2014 and 2015 combined with totals of unidentified isolates

	Genus and species identified (Synonym)		Genus and species identified (Synonym)
1	*Alternaria alternata*	57	*Fusarium tricinctum*
2	*Alternaria alternata/arborescens*	58	*Fusarium verticillioides/proliferatum*
3	*Alternaria alternata/tenuissima*	59	*Fusarium verticillioides*
4	*Alternaria arborescens*	60	*Fusarium verticillioides/sacchari*
5	*Alternaria brassicae*	61	*Fusarium verticillioides/sambucinum*
6	*Alternaria brassicae/alternata*	62	*Fusarium verticillioides/sterilihyphosum*
7	*Alternaria chartarum (Ulocladium chartarum)*	63	*Gaeumannomyces* sp.
8	*Alternaria consortiale*	64	*Gaeumannomyces radicicola*
9	*Alternaria infectoria*	65	*Harpophora zeicola*
10	*Alternaria infectoria/rosae*	66	*Lecanicillium lecanii*
11	*Alternaria tenuissima*	67	*Malassezia restricta*
12	*Ascochyta pinodes*	68	*Microdochium bolleyi*
13	*Ascochyta pinodes/fabae*	69	*Mucor circinelloides*
14	*Aspergillus ochraceus*	70	*Mucor fragilis*
15	*Beauveria bassiana*	71	*Mucor hiemalis*
16	*Bionectria ochroleuca*	72	*Mucor racemosus*
17	*Botrytis cinerea*	73	*Nigrospora oryzae*
18	*Botrytis fabae/cinerea*	74	*Paraphaeosphaeria michotii* (*Leptosphaeria michotii*)
19	*Candida sake*	75	*Paraphaeosphaeria sporulosa*
20	*Chaetomium globosum*	76	*Penicillium adametzioides/spinulosum*
21	*Chaetomium/Trichocladium*	77	*Penicillium adametzioides*
22	*Cladosporium colocasiae*	78	*Penicillium brasilianum*
23	*Cladosporium allicinum/herbarum*	79	*Penicillium chrysogenum*
24	*Cladosporium cladosporioides*	80	*Penicillium citreonigrum*
25	*Cladosporium sinuosum*	81	*Penicillium glabrum/adametzioides/spinulosum*
26	*Cladosporium* sp.	82	*Penicillium griseofulvum*
27	*Cladosporium tenuissimum*	83	*Penicillium purpurogenum*
28	*Cochliobolus intermedius/Curvularia trifolii*	84	*Penicillium* sp.
29	*Curvularia trifolii*	85	*Penicillium spinulosum*
30	*Didymella* sp.	86	*Pestalotiopsis disseminata*
31	*Drechslera dematioidea*	87	*Phoma glomerata*
32	*Drechslera* sp.	88	*Phoma herbarum*
33	*Epicoccum nigrum*	89	*Phoma paspali*
34	*Fusarium avenaceum*	90	*Phoma pinodella*
35	*Fusarium avenaceum/acuminatum*	91	*Phoma* sp.
36	*Fusarium cerealis/culmorum*	92	*Pithomyces chartarum*
37	*Fusarium konzum*	93	*Pyronema domesticum*
38	*Fusarium culmorum*	94	*Pythium aristosporum/arrhenomanes*
39	*Fusarium equiseti*	95	*Rhizopus oryzae*
40	*Fusarium equiseti/incarnatum*	96	*Sarocladium zeae* (*Acremonium zeae*)

41	*Fusarium flocciferum*
42	*Fusarium graminearum*
43	*Fusarium lateritium/acuminatum*
44	*Fusarium lunulosporum*
45	*Fusarium oxysporum*
46	*Fusarium proliferatum*
47	*Fusarium proliferatum/disseminata*
48	*Fusarium proliferatum/oxysporum/verticillioides*
49	*Fusarium proliferatum/Pestalotiopsis disseminata*
50	*Fusarium proliferatum/sacchari*
51	*Fusarium proliferatum*
52	*Fusarium sacchari/verticillioides*
53	*Fusarium sambucinum/venenatum*
54	*Fusarium* sp.
55	*Fusarium sterilihyphosum*
56	*Fusarium succisae/proliferatum*
97	*Schizothecium fimbriatum*
98	*Sordaria fimicola*
99	*Stemphylium globuliferum*
100	*Stemphylium vesicarium*
101	*Trichocladium* sp.
102	*Trichoderma asperellum*
103	*Trichoderma atroviride*
104	*Trichoderma gamsii*
105	*Trichoderma hamatum*
106	*Trichoderma harzianum*
107	*Trichoderma koningiopsis*
108	*Trichoderma koningiopsis/atroviride*
109	*Trichoderma koningiopsis/gamsii*
110	*Trichoderma* sp.
111	*Wallemia sebi*
112	unknown 22 isolates

A.4 Recovered species identified per plant (yr-plant number)

14-1	14-2	14-3	14-4	14-5	14-6
Alternaria alternata	*Curvularia trifolii*	*Alternaria alternata*	*Alternaria alternata*	*Ascochyta pinodes*	*Alternaria alternata*
Bionectria ochroleuca	*Epicoccum nigrum*	*Epicoccum nigrum*	*Fusarium oxysporum*	*Epicocum nigrum*	*Curvularia trifolii*
Cladosporium colocasiae	*Lecanicillium lecanii*		*Phoma herbarum*	*Fusarium equiseti*	*Epicoccum nigrum*
Fusarium culmorum	*Mucor hiemalis*		*Trichoderma hamatum*	*Fusarium oxysporum*	*Fusarium equiseti*
Fusarium graminearum	*Sordaria fimicola*			*Mucor fragilis*	*Penicillium brasilianum*
				Rhizopus oryzae	*Penicillium citreonigrum*
				Trichoderma atroviride	

14-7	14-8	14-9	14-10	14-11	14-12
Beauveria bassiana	*Epicoccum nigrum*	*Alternaria brassicae*	*Alternaria infectoria*	*Alternaria infectoria*	*Alternaria alternata*
Fusarium equiseti	*Paraphaeosphaeria michoti*	*Alternaria arborescens*	*Fusarium avenaceum*	*Mucor hiemalis*	*Epicoccum nigrum*
Fusarium oxysporum	*Trichoderma asperellum*	*Alternaria infectoria*	*Fusarium flocciferum*	*Trichoderma hamatum*	*Fusarium oxysporum*
Fusarium proliferatum		*Botrytis cinerea*	*Fusarium graminearum*		*Fusarium sterilihyphosum*
Fusarium verticillioides		*Candida sake*	*Fusarium lunulosporum*		*Fusarium tricinctum*
Mucor hiemalis		*Fusarium avenaceum*	*Fusarium oxysporum*		*Fusarium verticillioides*
Pyronema domesticum		*Phoma glomerata*	*Microdochium bolleyi*		*Trichoderma hamatum*
Stemphylium vesicarium		*Stemphylium globuliferum*			*Trichoderma harzianum*
					Trichoderma koningiopsis

14-14	14-15	14-16	14-17	14-18	14-19
Fusarium proliferatum	*Aspergillus ochraceus*	*Aspergillus ochraceus*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium oxysporum*
Microdochium bolleyi	*Fusarium konzum*	*Fusarium oxysporum*	*Penicillium purpurogenum*	*Fusarium proliferatum*	*Mucor fragilis*
	Microdochium bolleyi	*Microdochium bolleyi*			*Mucor racemosus*
	Phoma herbarum	*Phoma herbarum*			*Wallemia sebi*
	Trichoderma koningiopsis	*Sarocladium zeae*			

14-20	14-21	14-22
Botrytis cinerea *Fusarium verticillioides* *Malassezia restricta* *Microdochium bolleyi*	*Fusarium equiseti*	*Botrytis cinerea*

15-1	15-2	15-3	15-4	15-5	15-6
Alternaria alternata *Alternaria arborescens* *Alternaria infectoria* *Cladosporium cladosporioides* *Fusarium oxysporum* *Fusarium proliferatum* *Pithomyces chartarum* *Rhizopus oryzae*	*Alternaria alternata* *Drechslera dematioidea* *Fusarium avenaceum* *Fusarium equiseti* *Fusarium graminearum* *Trichoderma gamsii*	*Alternaria arborescens* *Alternaria infectoria* *Cladosporium tenuissimum* *Epicoccum nigrum* *Fusarium oxysporum* *Mucor circinelloides*	*Drechslera dematioidea* *Fusarium graminearum* *Fusarium oxysporum* *Gaeumannomyces radicicola* *Harpophora zeicola* *Nigrospora oryzae* *Penicillium chrysogenum* *Phoma paspali* *Pithomyces chartarum*	*Cladosporium cladosporioides* *Fusarium oxysporum*	*Chaetomium globosum* *Epicoccum nigrum* *Fusarium avenaceum* *Penicillium spinulosum*

15-7	15-8	15-9	15-10	15-11	15-12
Fusarium graminearum	*Alternaria alternata* *Alternaria arborescens* *Alternaria infectoria* *Alternaria tenuissima* *Penicillium griseofulvum*	*Alternaria alternata* *Alternaria arborescens* *Alternaria infectoria* *Cladosporium spinulosum* *Fusarium avenaceum* *Fusarium equiseti* *Mucor hiemalis*	*Alternaria alternata* *Alternaria arborescens* *Fusarium avenaceum* *Fusarium graminearum* *Fusarium proliferatum*	*Alternaria alternata* *Alternaria arborescens* *Alternaria chartarum* *Drechslera dematioidea* *Fusarium oxysporum* *Fusarium proliferatum* *Penicillium adametzioides* *Stemphylium vesicarium*	*Alternaria infectoria* *Fusarium avenaceum* *Fusarium equiseti* *Fusarium oxysporum* *Fusarium proliferatum* *Microdochium bolleyi*

A.5 Summary of genera and species identified for Canterbury and Waikato regions

Canterbury	Waikato
Alternaria alternata	*Alternaria alternata*
Alternaria alternata/arborescens	*Alternaria arborescens*
Alternaria alternata/tenuissima	*Alternaria consortiale*
Alternaria arborescens	*Alternaria infectoria*
Alternaria brassicae	*Alternaria tenuissima*
Alternaria brassicae/alternata	*Ascochyta pinodes*
Alternaria chartarum (Ulocladium chartarum)	*Ascochyta pinodes/fabae*
Alternaria infectoria	*Bionectria ochroleuca*
Alternaria infectoria/rosae	*Cladosporium colocasiae*
Alternaria tenuissima	*Cladosporium cladosporioides*
Aspergillus ochraceus	*Cladosporium* sp.
Beauveria bassiana	*Cladosporium tenuissimum*
Botrytis cinerea	*Cochliobolus intermedius/Curvularia trifolii*
Botrytis fabae/cinerea	*Curvularia trifolii*
Candida sake	*Drechslera dematioidea*
Chaetomium globosum	*Epicoccum nigrum*
Chaetomium/Trichocladium	*Fusarium avenaceum*
Cladosporium allicinum/herbarum	*Fusarium culmorum*
Cladosporium sinuosum	*Fusarium equiseti*
Cladosporium sp.	*Fusarium equiseti/incarnatum*
Didymella sp.	*Fusarium graminearum*
Drechslera dematioidea	*Fusarium oxysporum*
Drechslera sp.	*Fusarium proliferatum*
Epicoccum nigrum	*Fusarium proliferatum/disseminata*
Fusarium avenaceum	*Fusarium proliferatum/oxysporum/verticillioides*
Fusarium avenaceum/acuminatum	*Fusarium proliferatum/sacchari*
Fusarium cerealis/culmorum	*Fusarium proliferatum/verticillioides*
Fusarium culmorum	*Fusarium sacchari/verticillioides*
Fusarium equiseti	*Fusarium sterilihyphosum*
Fusarium flocciferum	*Fusarium succisae/proliferatum*
Fusarium graminearum	*Fusarium tricinctum*
Fusarium konzum	*Fusarium verticillioides*
Fusarium lateritium/acuminatum	*Fusarium verticillioides/sacchari*
Fusarium lunulosporum	*Fusarium verticillioides/sterilihyphosum*
Fusarium oxysporum	*Gaeumannomyces* sp.
Fusarium proliferatum	*Gaeumannomyces radicicola*
Fusarium proliferatum	*Harpophora zeicola*
Fusarium proliferatum/Pestalotiopsis disseminata	*Lecanicillium lecanii*
Fusarium sambucinum/venenatum	*Mucor circinelloides*
Fusarium verticillioides/proliferatum	*Mucor fragilis*
Fusarium verticillioides/sambucinum	*Mucor hiemalis*

Malassezia restricta
Microdochium bolleyi
Mucor fragilis
Mucor hiemalis
Mucor racemosus
Paraphaeosphaeria michotii (*Leptosphaeria michotii*)
Paraphaeosphaeria sporulosa
Penicillium adametzioides/spinulosum
Penicillium adametzioides
Penicillium griseofulvum
Penicillium purpurogenum
Penicillium spinulosum
Pestalotiopsis disseminata
Phoma glomerata
Phoma herbarum
Pyronema domesticum
Pythium aristosporum/arrhenomanes
Sarocladium zeae (*Acremonium zeae*)
Schizothecium fimbriatum
Sordaria fimicola
Stemphylium globuliferum
Stemphylium vesicarium
Trichocladium sp.
Trichoderma asperellum
Trichoderma hamatum
Trichoderma koningiopsis/atroviride
Trichoderma koningiopsis/gamsii
Wallemia sebi

unknown- 14 isolates

Nigrospora oryzae
Penicillium brasilianum
Penicillium chrysogenum
Penicillium citreonigrum
Penicillium glabrum/adametzioides/spinulosum
Penicillium sp.
Phoma paspali
Phoma pinodella
Phoma sp.
Pithomyces chartarum
Rhizopus oryzae
Sordaria fimicola
Trichoderma atroviride
Trichoderma gamsii
Trichoderma harzianum
Trichoderma koningiopsis
Trichoderma koningiopsis/gamsii
Trichoderma sp.

unknown -8 isolates

A.6 Summary of isolates found identified by sequencing and morphology in each plant in both 2014 and 2015

year - plant number	ID by BLAST and UNITE	ID by Morphology	year- plant number	ID BLAST and UNITE	ID by Morphology
14-1	*Alternaria alternata* *Bionectria ochroleuca* *Cladosporium colocasiae* *Fusarium culmorum* *Fusarium graminearum* *Trichoderma* sp.	*Epicoccum nigrum* *Fusarium* *Fusarium* 2 spp. *Fusarium avenaceum* *Fusarium oxysporum* *Penicillium* *Trichoderma* 3 spp.	15-1	*Alternaria alternata* *Alternaria arborescens* *Alternaria infectoria* *Cladosporium cladosporioides* *Cladosporium* sp. *Fusarium oxysporum* *Fusarium proliferatum* *Pithomyces chartarum* *Rhizopus oryzae*	*Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Fusarium graminearum* *Fusarium oxysporum* *Trichoderma* 3 spp.
14-2	*Alternaria* sp. *Curvularia trifolii* *Epicoccum nigrum* *Lecanicillium lecanii* *Mucor hiemalis* *Sordaria fimicola*	*Drechslera* *Epicoccum nigrum* *Fusarium* 2 spp. *Fusarium oxysporum* *Penicillium* *Rhizopus* *Trichoderma*	15-2	*Alternaria alternata* *Drechslera dematioidea* *Fusarium avenaceum* *Fusarium equiseti* *Fusarium graminearum* *Trichoderma gamsii* unknown-3	*Alternaria* *Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Fusarium oxysporum*
14-3	*Alternaria alternata* *Epicoccum nigrum*	*Epicoccum nigrum* *Fusarium* 2 spp. *Penicillium* *Trichoderma*	15-3	*Alternaria arborescens* *Alternaria infectoria* *Cladosporium tenuissimum* *Epicoccum nigrum* *Fusarium oxysporum* *Mucor circinelloides* *Penicillium* sp.	*Alternaria* *Epicoccum nigrum* *Fusarium* *Fusarium graminearum* *Trichoderma* 2 spp.
14-4	*Alternaria alternata* *Fusarium oxysporum* *Phoma herbarum* *Trichoderma* *Trichoderma hamatum*	*Epicoccum nigrum* *Fusarium oxysporum* *Fusarium* *Penicillium* *Trichoderma*			
14-5	*Ascochyta pinodes* *Cochliobolus intermedius/Curvularia trifolii* *Epicocum nigrum* *Fusarium equiseti* *Fusarium equiseti/incarnatum*	*Alternaria* *Epicoccum nigrum* *Fusarium* *Fusarium equiseti/incarnatum* *Fusarium oxysporum*	15-4	*Drechslera dematioidea* *Fusarium graminearum* *Fusarium oxysporum* *Gaeumannomyces radicicola* *Gaeumannomyces* sp.	*Epicoccum nigrum* *Fusarium equiseti* *Trichoderma*

	Fusarium oxysporum *Mucor fragilis* *Rhizopus oryzae* *Trichoderma atroviride*	*Penicillium* *Trichoderma*
14-6	*Alternaria alternata* *Curvularia trifolii* *Epicoccum nigrum* *Fusarium equiseti* *Penicillium glabrum/adametzioides/spinulosum* *Penicillium brasilianum* *Penicillium citreonigrum* *Trichoderma koningiopsis/gamsii*	*Epicoccum nigrum* *Fusarium* *Penicillium* *Trichoderma*
14-7	*Beauveria bassiana* *Fusarium equiseti* *Fusarium oxysporum* *Fusarium proliferatum* *Fusarium verticillioides* *Mucor hiemalis* *Penicillium adametzioides/spinulosum* *Pyronema domesticum* *Stemphylium vesicarium*	*Trichoderma*
14-8	*Epicoccum nigrum* *Paraphaeosphaeria michoti* *Trichoderma asperellum*	*Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Mucor* *Trichoderma*
14-9	*Alternaria brassicae* *Alternaria arborescens* *Botrytis cinerea* *Candida sake* *Fusarium avenaceum*	*Epicoccum nigrum* *Fusarium* *Rhizopus* *Trichoderma*
	Harpophora zeicola *Nigrospora oryzae* *Penicillium chrysogenum* *Phoma paspali* *Pithomyces chartarum* unknown-3	
15-5	*Cladosporium cladosporioides* *Cladosporium sp.* *Fusarium oxysporum* *Fusarium proliferatum/verticillioides* unknown	*Fusarium graminearum* *Fusarium oxysporum* *Fusarium* *Trichoderma* 2 spp.
15-6	*Chaetomium globosum* *Epicoccum nigrum* *Fusarium avenaceum* *Penicillium spinulosum* unknown 4	*Epicoccum nigrum* *Fusarium* *Penicillium* *Trichoderma*
15-7	*Alternaria brassicae/alternata* *Fusarium graminearum*	*Epicoccum nigrum* *Fusarium graminearum* *Trichoderma*
15-8	*Alternaria alternata* *Alternaria arborescens* *Alternaria infectoria* *Alternaria tenuissima* *Cladosporium* sp. *Lewia infectoria* *Penicillium griseofulvum* unknown 2	*Alternaria* *Fusarium* *Fusarium equiseti* *Trichoderma*
15-9	*Alternaria alternata* *Alternaria arborescens* *Alternaria infectoria* *Cladosporium spinulosum*	*Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Fusarium graminearum*

	Lewia infectoria *Phoma glomerata* *Stemphylium globuliferum*			*Fusarium avenaceum* *Fusarium equiseti* *Fusarium* sp.	*Trichoderma*
14-10	*Alternaria alternata/tenuissima* *Fusarium avenaceum/acuminatum* *Fusarium avenaceum* *Fusarium flocciferum* *Fusarium graminearum* *Fusarium lateritium/acuminatum* *Fusarium lunulosporum* *Fusarium oxysporum* *Fusarium verticillioides/sambucinum* *Lewia infectoria* *Microdochium bolleyi* *Schizothecium* spp.	*Epicoccum nigrum* *Fusarium culmorum* *Fusarium oxysporum/verticillioides* *Mucor*		*Mucor hiemalis* unknown	
14-11	*Fusarium culmorum/graminearum* *Lewia infectoria* *Mucor hiemalis* *Trichoderma hamatum* *Trichoderma koningiopsis/atroviride*	*Epicoccum nigrum* *Fusarium* *Mucor* *Trichoderma*	15-10	*Alternaria alternata* *Alternaria arborescens* *Drechslera* sp. *Fusarium avenaceum* *Fusarium graminearum* *Fusarium proliferatum* unknown	*Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Fusarium graminearum* *Fusarium oxysporum* *Trichoderma* 2 spp.
14-12	*Alternaria alternata* *Epicoccum nigrum* *Fusarium oxysporum* *Fusarium proliferatum/sacchari* *Fusarium proliferatum/andiyazi*	*Epicoccum nigrum* *Fusarium avenaceum* *Fusarium* *Fusarium oxysporum* *Mucor hiemalis*	15-11	*Alternaria alternata* *Alternaria arborescens* *Alternaria chartarum* *Drechslera dematioidea* *Fusarium oxysporum* *Fusarium proliferatum* *Penicillium adametzioides* *Stemphylium vesicarium* unknown	*Alternaria* *Fusarium* *Fusarium graminearum* *Fusarium oxysporum* *Rhizopus* *Trichoderma*
			15-12	*Alternaria infectoria* *Fusarium avenaceum* *Fusarium equiseti* *Fusarium oxysporum*	*Epicoccum nigrum* *Fusarium* *Fusarium equiseti* *Fusarium graminearum*

	Fusarium proliferatum/verticillioides *Fusarium sterilihyphosum* *Fusarium sterilihyphosum/proliferatum* *Fusarium tricinctum* *Fusarium verticillioides* *Trichoderma hamatum* *Trichoderma harzianum* *Trichoderma koningiopsis*	*Trichoderma* *Trichoderma koningiopsis/gamsii*
14-13		*Fusarium*
14-14	*Fusarium proliferatum* *Microdochium bolleyi*	*Fusarium* *Fusarium verticillioides*
14-15	*Aspergillus ochraceus* *Fusarium konzum* *Fusarium* sp. *Microdochium bolleyi* *Phoma herbarum* *Trichoderma koningiopsis*	*Fusarium* *Trichoderma* *Trichoderma*
14-16	*Aspergillus ochraceus* *Fusarium cerealis/culmorum* *Fusarium oxysporum* *Fusarium sambucinum/venenatum* *Microdochium bolleyi* *Phoma herbarum* *Sarocladium zeae*	*Epicoccum* *Fusarium* *Trichoderma*
14-17	*Fusarium oxysporum* *Penicillium purpurogenum*	*Epicoccum* *Fusarium* *Trichoderma*
14-18	*Fusarium equiseti* *Fusarium proliferatum*	*Epicoccum* *F. graminearum*
14-19	*Fusarium oxysporum* *Mucor fragilis*	*Epicoccum* *Fusarium* 2 spp.
	Fusarium proliferatum *Microdochium bolleyi* *Trichocladium* sp. unknown	*Fusarium oxysporum* *Penicillium* *Trichoderma* 2 spp.

	Mucor racemosus *Wallemia sebi*	*Penicillium*
14-20	*Botrytis cinerea* *Fusarium verticillioides* *Lewia infectoria/Alternaria rosae* *Malassezia restricta* *Microdochium bolleyi*	*Epicoccum*
14-21	*Fusarium equiseti* *Fusarium* sp. *Pythium aristosporum/arrhenomanes*	*Alternaria* *Epicoccum* *Fusarium oxysporum* *Fusarium*
14-22	*Botrytis cinerea* *Botrytis fabae/cinerea*	*Penicillium* *Trichoderma*

A.7 Location on the plant (2014 and 2015 seasons)

Location and site on plant	Genus and species identified (Synonym)
Roots	
Brace	*Alternaria alternata*
	Cladosporium allicinum/herbarum
	Fusarium avenaceum/acuminatum
	Fusarium cerealis/culmorum
	Fusarium equiseti
	Fusarium flocciferum
	Fusarium graminearum
	Fusarium lunulosporum
	Fusarium oxysporum
	Fusarium proliferatum
	Fusarium proliferatum/oxysporum/verticillioides
	Fusarium proliferatum/sacchari
	Fusarium verticillioides
	Fusarium verticillioides/sacchari
	Microdochium bolleyi
	Mucor racemosus
	Penicillium brasilianum
	Penicillium purpuroquenum
	Rhizopus oryzae
	Sarocladium zeae (*Acremonium zeae*)
	Trichoderma hamatum
	unknown 3 isolates
Seminal	*Alternaria alternata*
	Alternaria arborescens
	Alternaria chartarum (Ulocladium chartarum)
	Bionectria ochroleuca
	Chaetomium globosum
	Drechslera sp.
	Epicoccum nigrum
	Fusarium avenaceum
	Fusarium equiseti
	Fusarium equiseti/incarnatum
	Fusarium oxysporum
	Gaeumannomyces sp.
	Gaeumannomyces radicicola
Lower stem	
Top leaf	*Alternaria alternata*
	Alternaria arborescens
	Alternaria infectoria
	Alternaria tenuissima
	Botrytis cinerea
	Chaetomium/Trichocladium
	Epicoccum nigrum
	Fusarium equiseti
	Fusarium graminearum
	Fusarium oxysporum
	Fusarium proliferatum/disseminata
	Fusarium sacchari/verticillioides
	Fusarium sterilihyphosum
	Fusarium verticillioides
	Fusarium verticillioides/sambucinum
	Malassezia restricta
	Microdochium bolleyi
	Paraphaeosphaeria michotii (*Leptosphaeria michotii*)
	Phoma glomerata
	Phoma herbarum
	Rhizopus oryzae
	Trichocladium sp.
	unknown 2 isolates
Bottom leaf	*Alternaria alternata*
	Alternaria arborescens
	Alternaria brassicae/alternata
	Ascochyta pinodes
	Ascochyta pinodes/fabae
	Botrytis cinerea
	Drechslera dematioidea
	Epicoccum nigrum
	Fusarium avenaceum
	Fusarium equiseti
	Penicillium griseofulvum
	Phoma paspali

	Harpophora zeicola
	Mucor hiemalis
	Penicillium spinulosum
	Trichoderma hamatum
	Trichoderma koningiopsis/atroviride
	unknown 3 isolates
Radicle	*Alternaria alternata*
	Bionectria ochroleuca
	Botrytis cinerea
	Cladosporium colocasiae
	Drechslera sp.
	Fusarium avenaceum
	Fusarium culmorum
	Fusarium equiseti
	Fusarium graminearum
	Fusarium oxysporum
	Fusarium proliferatum
	Fusarium sp.
	Fusarium tricinctum
	Fusarium verticilliodes/proliferatum
	Microdochium bolleyi
	Mucor hiemalis
	Penicillium sp.
	Pestalotiopsis disseminata
	Rhizopus oryzae
	Trichoderma asperellum
	Trichoderma harzianum
	Trichoderma koningiopsis/gamsii
	Trichoderma sp.
	unknown

	Phoma pinodella
	Pithomyces chartarum
	Stemphylium vesicarium
	unknown 2 isolates
Stem	*Ascochyta pinodes*
	Cochliobolus intermedius/Curvularia trifolii
	Fusarium sp.

Location and site on plant	**Genus and species identified**
Ear	
Leaf husk	*Alternaria alternata*
	Alternaria alternata/tenuissima
	Alternaria brassicae
	Alternaria consortiale (Ulocladium consortiale)
	Alternaria infectoria
	Alternaria tenuissima
	Beauveria bassiana
	Candida sake
	Cladosporium sinuosum
	Cladosporium tenuissimum

Location and site on plant	**Genus and species identified**
Upper stem	
Top leaf	*Alternaria alternata*
	Alternaria arborescens
	Cladosporium cladosporioides
	Cladosporium sp.
	Epicoccum nigrum
	Fusarium graminearum
	Alternaria infectoria/Alternaria rosae
	Mucor circinelloides
	Nigrospora oryzae
	Penicillium sp.

	Curvularia trifolii
	Epicoccum nigrum
	Fusarium culmorum
	Fusarium oxysporum
	Fusarium sp.
	Microdochium bolleyi
	Mucor fragilis
	Penicillium adametzioides/spinulosum
	Penicillium adametzioides
	Pythium aristosporum/arrhenomanes
	Stemphylium globuliferum
	Trichoderma atroviride
	Trichoderma gamsii
	Trichoderma koningiopsis/gamsii
	unknown 3 isolates
Kernel	*Alternaria arborescens*
	Botrytis cinerea
	Botrytis fabae/cinerea
	Fusarium oxysporum
	Fusarium verticillioides
	Lecanicillium lecanii
	Mucor hiemalis
	Phoma herbarum
Silk	*Alternaria alternata/arborescens*
	Aspergillus ochraceus
	Cladosporium cladosporioides
	Cladosporium sp.
	Didymella sp.
	Fusarium oxysporum
	Fusarium oxysporum
	Fusarium proliferatum
	Fusarium sp.
	Paraphaeosphaeria sporulosa
	Penicillium citreonigrum
	Penicillium glabrum/adametzioides/spinulosum
	Phoma herbarum
	Pyronema domesticum
	Schizothecium fimbriatum
	Sordaria fimicola
	Stemphylium vesicarium
	Trichoderma gamsii
	Wallemia sebi
	unknown 4 isolates
	Phoma herbarum
	unknown 2 spp.
Bottom leaf	*Alternaria alternata*
	Alternaria arborescens
	Alternaria infectoria
	Alternaria tenuissima
	Cladosporium sp.
	Drechslera dematioidea
	Epicoccum nigrum
	Fusarium avenaceum
	Fusarium equiseti
	Fusarium graminearum
	Mucor circinelloides
	Penicillium chrysogenum
	Phoma sp.
	Pithomyces chartarum
	unknown
Stem	*Alternaria infectoria*
	Botrytis cinerea
	Epicoccum nigrum
	Fusarium avenaceum
	Fusarium equiseti
	Fusarium lateritium/acuminatum
	Fusarium oxysporum
	Fusarium proliferatum
	Fusarium proliferatum/verticillioides
	Mucor fragilis
	Trichoderma harzianum
	Trichoderma koningiopsis
	Trichoderma koningiopsis/atroviride
Tassel	*Alternaria infectoria*
	Botrytis fabae/cinerea
	Curvularia trifolii
	Epicoccum nigrum
	Fusarium avenaceum
	Fusarium sambucinum/venenatum
	Lewia infectoria
	Malassezia restricta
	Mucor hiemalis
Control	*Mucor racemosus*

Peduncle	*Cladosporium* sp.
	Fusarium konzum
	Fusarium oxysporum
	Fusarium proliferatum/verticillioides
	Fusarium sterilihyphosum
	Fusarium succisae/proliferatum
	Fusarium tricinctum
	Fusarium verticillioides/sterilihyphosum
	Mucor circinelloides
	Sordaria fimicola
	unknown 1

A.8 Summary of species found in main parts of the plant

Roots	Ear	Leaf	Stem	Tassel
Alternaria alternata	*Alternaria alternata*	*Alternaria alternata*	*Alternaria infectoria*	*Alternaria infectoria*
Alternaria arborescens	*Alternaria arborescens*	*Alternaria arborescens*	*Ascochyta pinodes*	*Botrytis fabae/cinerea*
Alternaria chartarum (Ulocladium chartarum)	*Alternaria brassicae*	*Alternaria brassicae/alternata*	*Botrytis cinerea*	*Curvularia trifolii*
Bionectria ochroleuca	*Alternaria consortiale*	*Alternaria infectoria*	*Cochliobolus intermedius/Curvularia trifolii*	*Epicoccum nigrum*
Botrytis cinerea	*Alternaria infectoria*	*Alternaria tenuissima*	*Epicoccum nigrum*	*Fusarium avenaceum*
Chaetomium globosum	*Alternaria tenuissima*	*Ascochyta pinodes*	*Fusarium avenaceum*	*Fusarium sambucinum/venenatum*
Cladosporium colocasiae	*Aspergillus ochraceus*	*Ascochyta pinodes/fabae*	*Fusarium equiseti*	*Malassezia restricta*
Cladosporium allicinum/herbarum	*Beauveria bassiana*	*Botrytis cinerea*	*Fusarium lateritium/acuminatum*	*Mucor hiemalis*
Drechslera sp.	*Botrytis cinerea*	*Chaetomium/Trichocladium*	*Fusarium oxysporum*	8
Epicoccum nigrum	*Candida sake*	*Cladosporium cladosporioides*	*Fusarium proliferatum*	
Fusarium avenaceum	*Cladosporium cladosporioides*	*Cladosporium* sp.	*Fusarium proliferatum/verticillioides*	
Fusarium avenaceum/acuminatum	*Cladosporium sinuosum*	*Drechslera dematioidea*	*Mucor fragilis*	
Fusarium cerealis/culmorum	*Cladosporium tenuissimum*	*Epicoccum nigrum*	*Pithomyces chartarum*	
Fusarium culmorum	*Curvularia trifolii*	*Fusarium avenaceum*	*Trichoderma harzianum*	
Fusarium equiseti	*Didymella* sp.	*Fusarium equiseti*	*Trichoderma koningiopsis*	
Fusarium equiseti/incarnatum	*Epicoccum nigrum*	*Fusarium graminearum*	*Trichoderma koningiopsis/atroviride*	
Fusarium flocciferum	*Fusarium konzum*	*Fusarium oxysporum*	16	
Fusarium graminearum	*Fusarium culmorum*	*Fusarium proliferatum/disseminata*		
Fusarium lunulosporum	*Fusarium oxysporum*	*Fusarium sacchari/verticillioides*		
Fusarium oxysporum	*Fusarium proliferatum*	*Fusarium sterilihyphosum*		
Fusarium proliferatum	*Fusarium proliferatum/verticillioides*	*Fusarium verticilliodes/sambucinum*		
Fusarium proliferatum/verticillioides	*Fusarium sterilihyphosum*	*Fusarium verticillioides*		

Fusarium proliferatum/sacchari	*Fusarium succisae/proliferatum*	*Lewia infectoria/Alternaria rosae*
Fusarium tricinctum	*Fusarium tricinctum*	*Malassezia restricta*
Fusarium verticilliodes/proliferatum	*Fusarium verticillioides*	*Microdochium bolleyi*
Fusarium verticillioides	*Lecanicillium lecanii*	*Mucor circinelloides*
Fusarium verticillioides/sacchari	*Microdochium bolleyi*	*Nigrospora oryzae*
Gaeumannomyces sp.	*Mucor circinelloides*	*Paraphaeosphaeria michoti*
Gaeumannomyces radicicola	*Mucor fragilis*	*Penicillium chrysogenum*
Harpophora zeicola	*Mucor hiemalis*	*Penicillium griseofulvum*
Microdochium bolleyi	*Paraphaeosphaeria sporulosa*	*Phoma glomerata*
Mucor hiemalis	*Penicillium glabrum/adametzioides/spinulosum*	*Phoma herbarum*
Mucor racemosus	*Penicillium adametzioides*	*Phoma paspali*
Penicillium brasilianum	*Penicillium citreonigrum*	*Phoma pinodella*
Penicillium purpurogenum	*Phoma herbarum*	*Pithomyces chartarum*
Penicillium spinulosum	*Pyronema domesticum*	*Rhizopus oryzae*
Pestalotiopsis disseminata	*Pythium aristosporum/arrhenomanes*	*Stemphylium vesicarium*
Rhizopus oryzae	*Schizothecium fimbriatum*	*Trichocladium* sp.
Sarocladium zeae	*Sordaria fimicola*	
Trichoderma asperellum	*Stemphylium globuliferum*	
Trichoderma hamatum	*Stemphylium vesicarium*	
Trichoderma harzianum	*Trichoderma atroviride*	
Trichoderma koningiopsis/atroviride	*Trichoderma gamsii*	
Trichoderma koningiopsis/gamsii	*Trichoderma koningiopsis/gamsii*	
	Wallemia sebi	

44 45 38

A.9 All data combined from leaf and stem locations to assess as site specific endophytes

Species isolates from leaves from all locations of the maize plant

Leaves	Stems
Alternaria alternata	*Alternaria infectoria*
Alternaria arborescens	*Ascochyta pinodes*
Alternaria brassicae	*Botrytis cinerea*
Alternaria consortiale (Ulocladium consortiale)	*Cochliobolus intermedius/Curvularia trifolii*
Alternaria infectoria	*Epicoccum nigrum*
Alternaria infectoria/rosae	*Fusarium avenaceum*
Alternaria tenuissima	*Fusarium equiseti*
Ascochyta pinodes	*Fusarium lateritium/acuminatum*
Ascochyta pinodes/fabae	*Fusarium oxysporum*
Beauveria bassiana	*Fusarium proliferatum*
Botrytis cinerea	*Fusarium proliferatum/verticillioides*
Candida sake	*Fusarium* sp.
Chaetomium/Trichocladium	*Mucor fragilis*
Cladosporium cladosporioides	*Trichoderma harzianum*
Cladosporium sinuosum	*Trichoderma koningiopsis*
Cladosporium sp.	*Trichoderma koningiopsis/atroviride*
Cladosporium tenuissimum	
Curvularia trifolii	
Drechslera dematioidea	
Epicoccum nigrum	
Fusarium avenaceum	
Fusarium culmorum	
Fusarium equiseti	
Fusarium graminearum	
Fusarium oxysporum	
Fusarium proliferatum/disseminata	
Fusarium sacchari/verticillioides	
Fusarium sterilihyphosum	
Fusarium verticillioides	
Fusarium verticillioides/sambucinum	
Malassezia restricta	
Microdochium bolleyi	
Mucor circinelloides	
Mucor fragilis	
Nigrospora oryzae	
Paraphaeosphaeria michotii (*Leptosphaeria michotii*)	
Penicillium adametzioides	

Penicillium adametzioides/spinulosum
Penicillium chrysogenum
Penicillium griseofulvum
Phoma glomerata
Phoma herbarum
Phoma paspali
Phoma pinodella
Pithomyces chartarum
Pythium aristosporum/arrhenomanes
Rhizopus oryzae
Stemphylium globuliferum
Stemphylium vesicarium
Trichocladium sp.
Trichoderma atroviride
Trichoderma gamsii
Trichoderma koningiopsis/gamsii

A.10 Specific location of recovery for genera (number of isolates shown)

		Roots			Lower stem			Ear				Upper stem				
Year	Genera	brace	seminal	radicle	top leaf	bottom leaf	stem	leaf husk	kernel	silk	peduncle	top leaf	stem	tassel	bottom leaf	totals identified
2014	*Alternaria*			1	2	3		11				2				19
2015	*Alternaria*	1	3		4	3		1	1	1		5	3		7	29
2014	*Ascochyta*					2	1									3
2014	*Aspergillus*									2						2
2014	*Beauveria*							2								2
2014	*Bionectria*		1	1												2
2014	*Botrytis*							1	2				1	1		5
2015	*Botrytis*			2	1	1										4
2014	*Candida*							1								1
2015	*Chaetomium*		1		1											2
2014	*Cladosporium*			1												1
2015	*Cladosporium*	1						2		2	1	2			1	9
2014	*Cochliobolus*					1	1									2
2014	*Curvularia*							1					3			4
2014	*Didymella*									1						1
2015	*Drechslera*		1	3		1									3	8
2014	*Epicoccum*				2	1		4				1	1	1		10
2015	*Epicoccum*		1	1											1	3
2014	*Fusarium*	14	6	11	8		1	3	2	4	5		10	2		66
2015	*Fusarium*	9	7	8	5	2					1	2	1		4	39
2015	*Gaeumannomyces*		2													2
2015	*Harpophora*		2													2
2014	*Lecanicillium*								1							1
2014	*Malassezia*				1									1		2
2014	*Microdochium*	1		1	2		1	1								6

2015	*Microdochium*					1													1
2014	*Mucor*	1		1					1		1				1		1		7
2015	*Mucor*				2							1		1				1	5
2015	*Nigrospora*													2					2
2014	*Paraphaeosphaeria*					1													1
2015	*Paraphaeosphaeria*										1								1
2014	*Penicillium*	2							1		2								5
2015	*Penicillium*			1	1		1		1					1				1	6
2014	*Pestalotiopsis*				1														1
2014	*Phoma*					2	1			1	1			1					6
2015	*Phoma*						1											1	2
2015	*Pithomyces*						1											1	2
2014	*Pyronema*										1								1
2014	*Pythium*								1										1
2014	*Rhizopus*	1																	1
2015	*Rhizopus*				1	1													2
2014	*Sarocladium*	1																	1
2014	*Schizothecium*										1								1
2014	*Sordaria*										1								1
2015	*Sordaria*											1							1
2014	*Stemphylium*								2		1								3
2015	*Stemphylium*							1											1
2015	*Trichocladium*					1													1
2014	*Trichoderma*	1		4	4				2						3				14
2015	*Trichoderma*				3				1		1								5
2014	*Wallemia*										1								1
2014	unknown			1															1
2015	unknown	3		2	1	2	2		3		4	1		2		1			21

Appendix B Dual culture

B.1 Isolates used in dual culture with growth averages for pathogen and isolates

Number of isolates	Isolate name	Pathogen growth average (cm)	Isolate average growth (cm)
c	control	3.16	3.81
1	*95-3richoderma* sp.	0.50	8.00
2	*119-Peniciiluim brasilianum*	0.50	7.67
3	*95-7-Trichoderma harzianum*	0.62	8.00
4	*36-Sordaria fimicola*	0.89	8.00
5	*95-6-Trichoderma hamatum*	1.13	6.00
6	*95-9-Trichoderma koningiopsis*	1.17	8.00
7	*108-Penicillium purpurogenum*	1.20	8.00
8	*55-Trichoderma hamatum*	1.23	8.00
9	*85A-Trichoderma koningiopsis/atroviride*	1.27	8.00
10	*95-10-Trichoderma asperellum*	1.33	8.00
11	*95-5-Trichoderma hamatum*	1.35	8.00
12	*137-Wallemia sebi*	1.58	6.62
13	*46-Trichoderma koningiopsis/gamsii*	1.59	8.00
14	*95-8-Trichoderma atroviride*	1.63	8.00
15	*3-Rhizopus oryzae*	1.64	8.00
16	*95-2Trichoderma koningiopsis/gamsii*	1.73	8.00
17	*82B-Fusarium avenaceum*	1.78	3.18
18	*125-Aspergillus ochraceus*	1.83	2.28
19	*99-Epicoccum nigrum*	1.85	5.23
20	*130-Botrytis cinerea*	1.85	6.10
21	*73-Fusarium avenaceum*	1.93	2.09
22	*118-Mucor fragilis*	2.02	8.00
23	*69-Alternaria alternata*	2.07	5.68
24	*152-Stemphylium globuliferum*	2.12	3.95
25	*157-Fusarium flocciferum*	2.18	2.58
26	*86-Lecanicillium lecanii*	2.19	2.69
27	*107-Phoma herbarum*	2.20	6.12
28	*105-Phoma herbarum*	2.23	6.43
29	*143-Trichoderma koningiopsis/atroviride*	2.24	7.67
30	*138-Malassezia restricta*	2.25	5.78
31	*38l-Alternaria consortiale*	2.27	4.99
32	*25-Epicoccum nigrum*	2.31	4.08
33	*38d-Alternaria alternata*	2.38	4.78
34	*145-Fusarium oxysporum*	2.38	7.14
35	*80-Penicillium olsonii*	2.42	1.59
36	*35A-Fusarium proliferatum*	2.44	6.39

37	4-*Alternaria alternata*	2.55	6.16
38	*102a-Phoma herbarum*	2.58	6.12
39	*134-Penicillium adametzioides/spinulosum*	2.63	6.15
40	*59-Fusarium verticillioides/sterilihyphosum*	2.73	6.48
41	*19-Mucor fragilis*	2.74	7.50
42	*132-Cochliobolus intermedius*	2.77	6.02
43	*50-Curvularia trifolii*	2.90	7.03
44	*129-Fusarium equiseti*	2.97	5.54
45	*42-Botrytis fabae/cinerea*	3.00	8.00
46	*53a-Fusarium lateritium/acuminatum*	3.03	3.98
47	12-*Alternaria alternata*	3.08	5.34
48	*7-Fusarium oxysporum*	3.13	6.20
49	*148-Aletrnaria infectoria*	3.18	3.17
50	*30b-Fusarium avenaceum/acuminatum*	3.20	3.17
51	*45d-Fusarium verticillioides*	3.22	5.88
52	*32-Fusarium verticillioides*	3.27	6.92
53	8c-*Fusarium sp.*	3.34	5.33
54	*101-Fusarium sambucinum/venenatum*	3.35	5.01
55	15-*Mucor hiemalis*	3.46	7.56
56	6-*Ascochyta pinodes/fabae*	3.51	5.38
57	*123-Fusarium equiseti*	3.66	5.79
58	*89-Fusarium tricinctum*	3.71	5.38
59	*28-Epicoccum nigrum*	3.75	5.80
60	*127-Didymella* sp.	3.78	5.00
61	*144-Fusarium oxysporum*	3.89	5.29
62	*37-Fusarium sterilihyphosum*	3.89	6.81
63	*26-Epicoccum nigrum*	3.95	3.68
64	*41-Alternaria alternata*	3.96	5.51
65	*133-Fusarium oxysporum*	4.00	6.18
66	13-*Bionectria ochroleuca*	4.08	3.30
67	9A*Fusarium graminearum*	4.11	4.54
68	*J21-Beauveria bassiana*	4.11	1.33
69	*24-Epicoccum nigrum*	4.23	2.76
70	*58-Fusarium avenaceum*	4.55	5.15
71	*J18-Beauveria bassiana*	4.59	2.76
72	*72-Fusarium flocciferum*	4.68	2.20
73	*47-Paraphaeosphaeria michotii*	4.71	3.46
74	*51-Fusarium proliferatum*	4.88	4.96
75	10-*Mucor hiemalis*	4.88	6.77
76	*114-Fusarium oxysporum*	4.96	5.31
77	*60A-Fusarium avenaceum*	5.11	3.59
78	*139-Fusarium oxysporum*	5.17	5.40
79	*141-Pythium aristosporum/arrhenomanes*	5.61	4.02

Appendix C *In planta*

C.1 Ratio of disease (NLB) score for fungal inoculated plants compared to control plants

Isolate number and name	cultivar	
	38V12	P0021
80-*Penicillium olsonii*	1.2	1.3
137B-*Wallemia sebi*	1.0	1.0
3-*Rhizopus oryzae*	1.3	1.4
15-*Mucor hiemalis*	0.9	1.6
53a-*Fusarium acuminatum*	0.9	1.0
105-*Phoma herbarum*	0.6	1.2
125-*Aspergillus ochraceus*	0.7	1.1
134-*Penicillium adametzioides/spinulosum*	0.5	1.3
86-*Lecanicillium lecanii*	1.0	0.9
19-*Mucor fragilis*	0.8	1.0
95-8-*Trichoderma atroviride*	1.0	0.7
129B-*Fusarium equiseti*	1.0	0.9
24-*Epicoccum nigrum*	1.0	0.9
108-*Penicillium purpurogenum*	0.8	1.7
50-*Curvularia trifolii*	1.1	1.6
43A-*Mucor racemosus*	0.5	0.7
36-*Sordaria fimicola*	0.6	0.6
J21-*Beauveria bassiana*	0.9	1.1
119-*Penicillium brasilianum*	0.7	0.9
51-*Fusarium proliferatum*	1.0	0.9
60A-*Fusarium avenaceum*	1.4	0.6
total	18.7	22.4
average	0.9	1.1

Appendix D-Literature review of identified species

D.1 Previous literature on known characteristics of species identified

Fungal Class	Genus & species identified (common synonym)	Recorded lifestyle	Disease caused	Other characteristics of note	Representative reference(s)
Phylum-Ascomycota Dothideomycetes	*Alternaria* spp.	Pathogen#		Mycotoxins Epiphytes	Logrieco *et al.* (2009) Stone *et al.* (2017)
	Alternaria alternata	Pathogen	Root rot, leaf spot, ear rot		Arzanlou *et al.* (2012)
	Alternaria alternata	Latent pathogen			Fisher and Petrini (1992)
	Alternaria arborescens	Pathogen	Leaf spot		Akhtar *et al.* (2014)
	Alternaria brassicae	Pathogen	Alternaria leaf spot		Conn *et al.* (1988) Chhikara *et al.* (2012)
	Alternaria infectoria				Logrieco *et al.* (2009)
	Alternaria tenuissima	Pathogen	Leaf spot		Arzanlou *et al.* (2012)
	Ascochyta pinodes	Pathogen	Ascochyta blight , Ascochyta foot rot		Le May *et al.* (2009)
	Ascochyta pinodes/fabae	Pathogen	Ascochyta blight		Román *et al.* (2003)
	Cladosporium sp.	Pathogen	Epiphyte		Ismaiel and Papenbrock (2015) Stone *et al.* (2017)
	Cladosporium cladosporioides	Pathogen	leaf spot, ear rot, seed rot		Arzanlou *et al.* (2012) Ismaiel & Papenbrock (2015) Abe *et al.* (2015)
	Cladosporium colocasiae	Pathogen			McKenzie (1990)
	Cladosporium tenuissimum	Beneficial^	BCA against pine stem rust	Plant growth regulator	Moricca *et al.* (2001)
	Cochliobolus intermedius (Curvularia intermedius)	Beneficial	BCA of crabgrass		Tilley and Walker (2002)

	Cochliobolus intermedius (Curvularia intermedius)	Pathogen	Curvularia leaf spot		Shurtleff *et al.* (1993)
	Curvularia sp.	Pathogen		Seed rot Seed endophyte	Abe *et al*. (2015) Zakaria *et al.* (2010)
	Curvularia trifolii	Beneficial			Rodriquez *et al*. (2009)
	Didymella sp.	Pathogen	Didymella leaf spot		Shurtleff *et al*. (1993)
	Drechslera dematioidea	Pathogen			Shurtleff *et al*. (1993)
	Epicoccum nigrum	Pathogen	Leaf spot, ear mould, leaf & seed rot	Saprophyte -weak parasite	Abe *et al*. (2015) Crocker *et al*. (2016)
	Paraphaeosphaeria michoti (Leptosphaeria michotii)	Pathogen	Leptosphaeria leaf spot		Wong *et al.* (2000) Shurtleff *et al*. (1993)
	Phoma glomerata	Pathogen			Maharachchikumbura *et al.* (2011) Johnston (1981)
	Phoma herbarum	Pathogen	Saprophyte	Toxigenic to humans	Hamayun *et al.* (2009)
	Phoma herbarum	Beneficial		Potential plant promoter, gibberellin	Hamayun *et al*. (2009)
	Phoma paspali	Pathogen			Johnstone (1981)
	Phoma pinodella	Pathogen	Leaf spot		Chen *et al.* (2015)
	Phoma sp.	Beneficial	Root endophyte	Plant growth promoter	Rodriguez *et al.* (2009) Hamayun *et al.* (2009)
	Pithomyces chartarum	Pathogen	Facial eczema on sheep		Brook (1962)
	Stemphylium globuliferum	Pathogen	Leaf spot		Samac D.A. (2014)
	Stemphylium globuliferum	Beneficial		Anti-cancer potential	Teiten *et al.* (2013)
	Stemphylium vesicarium	Pathogen	Leaf spot		Arzanlou *et al.* (2012)
Eurotiomycetes	*Aspergillus ochraceus*	Pathogen	Cob field-storage rot seed storage	Aflatoxin	Ismaiel & Papenbrock (2015)
	Aspergillus sp.	Seed pathogen		Seed rot	Abe *et al*. (2015)
	Penicillium adametzioides	Beneficial		BCA*	Ismaiel & Papenbrock (2015)
	Penicillium spp.	Pathogen	Seedling rot, ear rot		Shurtleff *et al*. (1993)
	Penicillium brasilianum	Pathogen			Sang *et al.* (2014)
	Penicillium chrysogenum	Beneficial		Source of medicinal Penicillin	Volk (2003)

	Penicillium citreonigrum	Pathogen		Mycotoxin	Rosa *et al.* (2008)
	Penicillium commune	Seed pathogen	Storage pathogen	Aflatoxin	Ismaiel & Papenbrock (2015)
	Penicillium griseofulvum	Pathogen		Food storage	Laich *et al.* (2002)
	Penicillium purpuroquenum	Beneficial		Absorption of heavy metals	Say *et al*. (2003)
	Penicillium raistrickii	Beneficial		Solubilizes calcium phosphate Produces griseofulvum antifungal	Seifert (1997-2017) Brian *et al.* (1955)
	Penicillium spinulosum	Pathogen	Food spoilage		Battley *et al*. 2001
Leotiomycetes	*Botrytis cinerea*	Pathogen	Ear rot		Fowler 1985
Saccharomycetes	*Candida sake*	Beneficial		BCA	Nunes *et al*. 2002
Sordariomycetes	*Beauveria bassiana*	Beneficial		BCA-Entomopathogenic fungus	Reay *et al.* 2010 Ownley *et al*. 2008 Wagner & Lewis 2000 And many others
	Bionectria ochroleuca	Beneficial		BCA-Entomopathogenic fungus	Guesmi-Jouini *et al*. 2014 Samaga *et al*. 2014
	Chaetomium globosum	Pathogen	Seed pathogen, seedling root rot?		Fowler 1985
	Fusarium sp.		Seed endophyte		Zakaria *et al*. 2010
	Fusarium avenaceum	Pathogen	Stalk rot, seedling root rot, seedling blight		Fowler 1985
	Fusarium acuminatum	Pathogen	Root rot		Logrieco *et al*. 1995
	Fusarium culmorum	Pathogen	Seed rot, seedling blight		Fowler 1985 Logrieco *et al*. 1995
	Fusarium culmorum	Beneficial			Rodriquez *et al.* 2011
	Fusarium equiseti (Gibberella intricans)	Latent pathogen			Fisher & Petrini 1992
	Fusarium equiseti	Pathogen	Root rot		Logrieco *et al*. 1995

Fusarium equiseti/incarnatum	Pathogen	Seed rot		Abe *et al.* 2015
Fusarium flocciferum	Pathogen			Miao *et al*. 2015 Chen *et al.* 2016a
Fusarium graminearum (Gibberella zea)	Pathogen	Ear & stalk rot, seed blight, head blight, seedling blight	Mycotoxins	Monds *et al*. 2005 Asran & Buchenauer 2003 and others
Fusarium konzum	Pathogen	Associated with *Giberella fujikuroi* complex-		Troncoso *et al*. 2010
Fusarium oxysporum	Pathogen	Stalk rot, Root rot	Soil saprophyte	Fravel *et al* 2002
Fusarium oxysporum	Latent pathogen			Fisher & Petrini 1992
Fusarium oxysporum	Beneficial		BCA	Rodriquez *et al*. 2010 Fravel *et al*. 2002,
Fusarium poae	Pathogen	Head blight, stalk rot, root rot		Leslie *et al.* 2008
Fusarium proliferatum	Pathogen	Kernel, root & stalk rots seed rot, seedling blight	Seed endophyte	Logrieco *et al*. 1995
Fusarium proliferatum	Pathogen		Human toxin	Ferrer *et al*. 2005
Fusarium sterilihyphosum	Pathogen			Leslie *et al*. 2008
Fusarium tricinctum	Saprophyte			Leslie *et al*.2008
Fusarium verticillioides (Fusarium moniliforme)	Pathogen	Kernel, root & stalk rots seed rot, seedling blight	Fumonisins (mycotoxin)	Atukwase *et al.* 2012 Eckard *et al*. 2011 Fowler 1985 and others
Fusarium verticillioides	Beneficial		Reduces *U. maydis* smut disease	Porras-Alfaro & Bayan 2011 Lee *et al*. 2009
Gaeumannomyces graminis	Pathogen	Take all' disease		Deacon 1973 Shurtleff *et al*. 1993
Gaeumannomyces radicicola	Pathogen			Luo & Zhang 2015.
Harpophora zeicola	Pathogen	Late wilt of maize	Root parasite	Deacon & Scott 1983
Lecanicillium lecanii	Beneficial	BCA	Entomopathogenic fungus	Vidal & Jaber 2015 Cortez-Madrigal *et al*.2003 Porras-Alfaro & Bayan 2011

	Microdochium bolleyi	Pathogen	Root rot, basal rot	Seed endophyte	Hong *et al.* 2008 Ernst *et al*. 2011
	Microdochium bolleyi	Beneficial			Lascaris & Deacon 1991.
	Nigrospora oryzae	Pathogen	Dry ear rot, crown rot,	Saprophyte	Kumar *et al*. 2015
	Nigrospora oryzae	Latent pathogen			Fisher & Petrini 1992
	Pestalotiopsis sp.	Pathogen	Leaf blight post-harvest rots	Weak pathogen affect stressed plants	Maharachchikumbura *et al*. 2011
	Pythium aristosporum/arrhenomanes		Root rot		Richter & Barnard 2002
	Sarocladium zeae (*Acremoniumn zeae*)	Pathogen	Sheath rot		Sakthivel *et al.* 2002 Abe *et al*. 2015
	Sordaria fimicola	Saprophyte		Dung fungi	Newcombe *et al*. 2016
	Trichocladium	Beneficial		Cytotoxic effect on cancer cells in humans	Guo *et al*. 2009
	Trichocladium sp.	Saprophyte			Seidl 2010 Goh & Hyde 1999
	Trichoderma asperellum	Beneficial	BCA		Maag *et al.* 2014
	Trichoderma atroviride	Beneficial	BCA		Maag *et al*. 2014
	Trichoderma gamsii	Beneficial	BCA	Stress tolerance	Chen *et al*. 2016a
	Trichoderma hamatum	Beneficial	BCA		Studholme *et al.* 2013
	Trichoderma harzianum	Pathogen		Seed rot	Abe *et al*. 2015
	Trichoderma harzianum	Beneficial	BCA against *Pythium*, increased seed germination & enhanced growth		Moran-Diez *et a*l. 2009 Shukla *et al*. 2012 Ahmad & Baker 1987
	Trichoderma koningiopsis	Beneficial	BCA		Chen *et al.* 2016b
Pezizomycetes (Ascomycetes)	*Pyronema domesticum*	Environmental mould	Toxic to humans		Aoshuang 1998 Richter & Barnard 2002
Phylum-Basidiomycota					
Exobasidiomycetes	*Malassezia restricta*	Pathogen		Human pathogen Skin disease	Gaitanis *et al*. 2012 Heitman 2011
Wallemiomycete (Hyphomycete)	*Wallemia sebi*	Pathogen	Storage fungi	Respiratory effect	Hanhela *et al*. 1995

Phylum-Zygomycota					
Zygomycetes	*Mucor circinelloides*	Pathogen		Food spoilage	Snyder *et al*. 2016
	Mucor circinelloides	Beneficial	Biodiesel		Grigoriev (1997-2017)
	Mucor fragilis	Pathogen	Stalk rot Root rot	Food storage, mycotoxin	Efuntoye 1996 Abe *et al*. 2015
	Mucor hiemalis	Pathogen		Food storage	Bonner & Fergus 1959 Werner & Zadworny 2003
	Mucor hiemalis	Beneficial		Removal of heavy metals	Srivastava & Hasan 2011
	Mucor racemosus	Pathogen			Thermo Scientific
Zygomycetes	*Rhizopus oryzae*	Saprophyte/pathogen	Food contamination	Human pathogen	Santiago & Motta 2007
	Rhizopus oryzae	Beneficial		Food additive in Tempeh Biodiesel	Cantarbrana *et al*. 2015 Ban et *al.* 2012
Superphylum-Heterokonta					
Oomycetes	*Pythium aristosporum/arrhenomanes*	Pathogen	Root rot		Richter & Barnard 2002

*BCA- biocontrol agent

#-plant pathogen

^- Beneficial in the context of this table refers to a benefit given to the host plant in an agriculture text.

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